

EXPERIMENTAL AND
RESEARCH STATION
CHESHUNT, HERTS

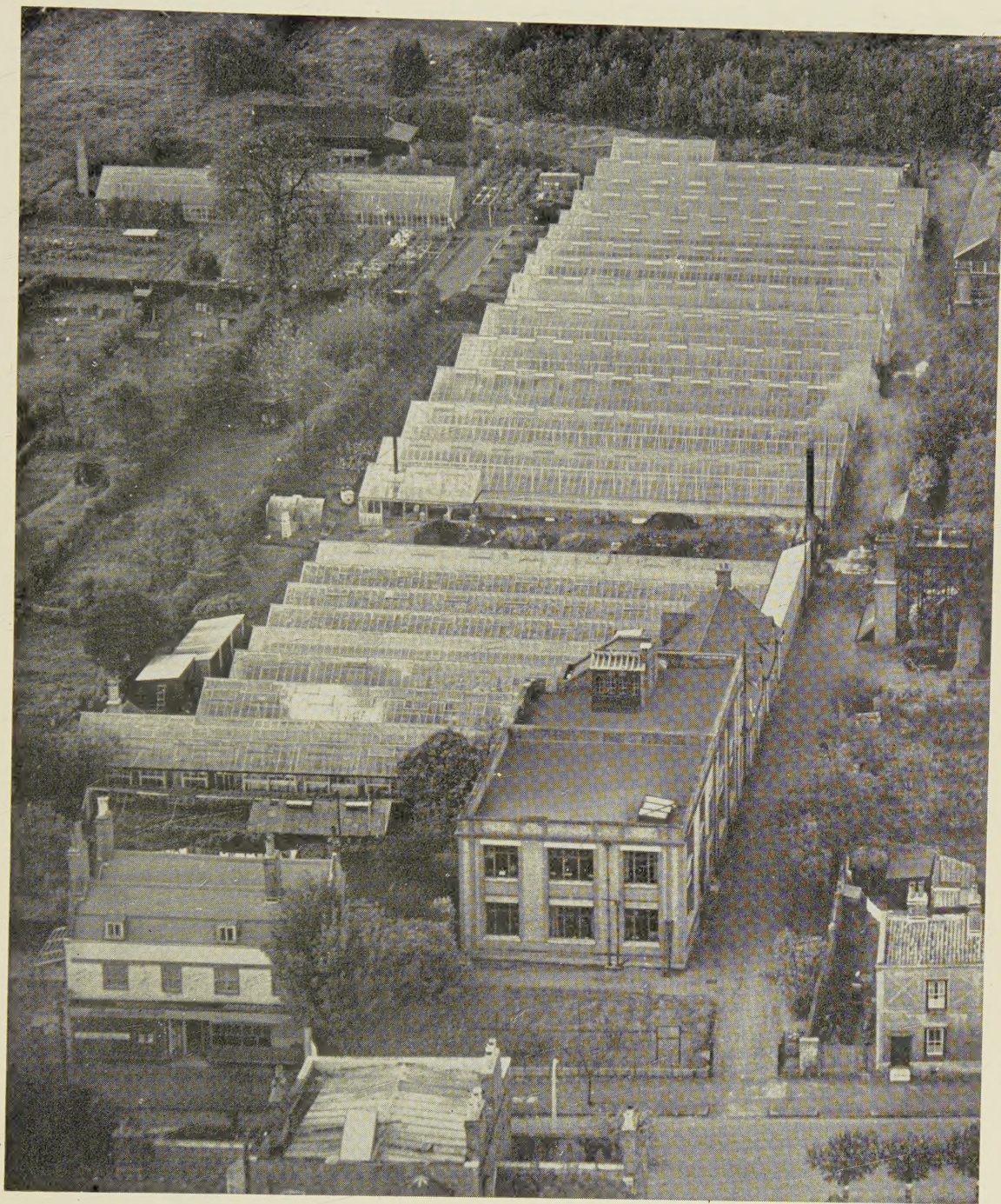
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1949

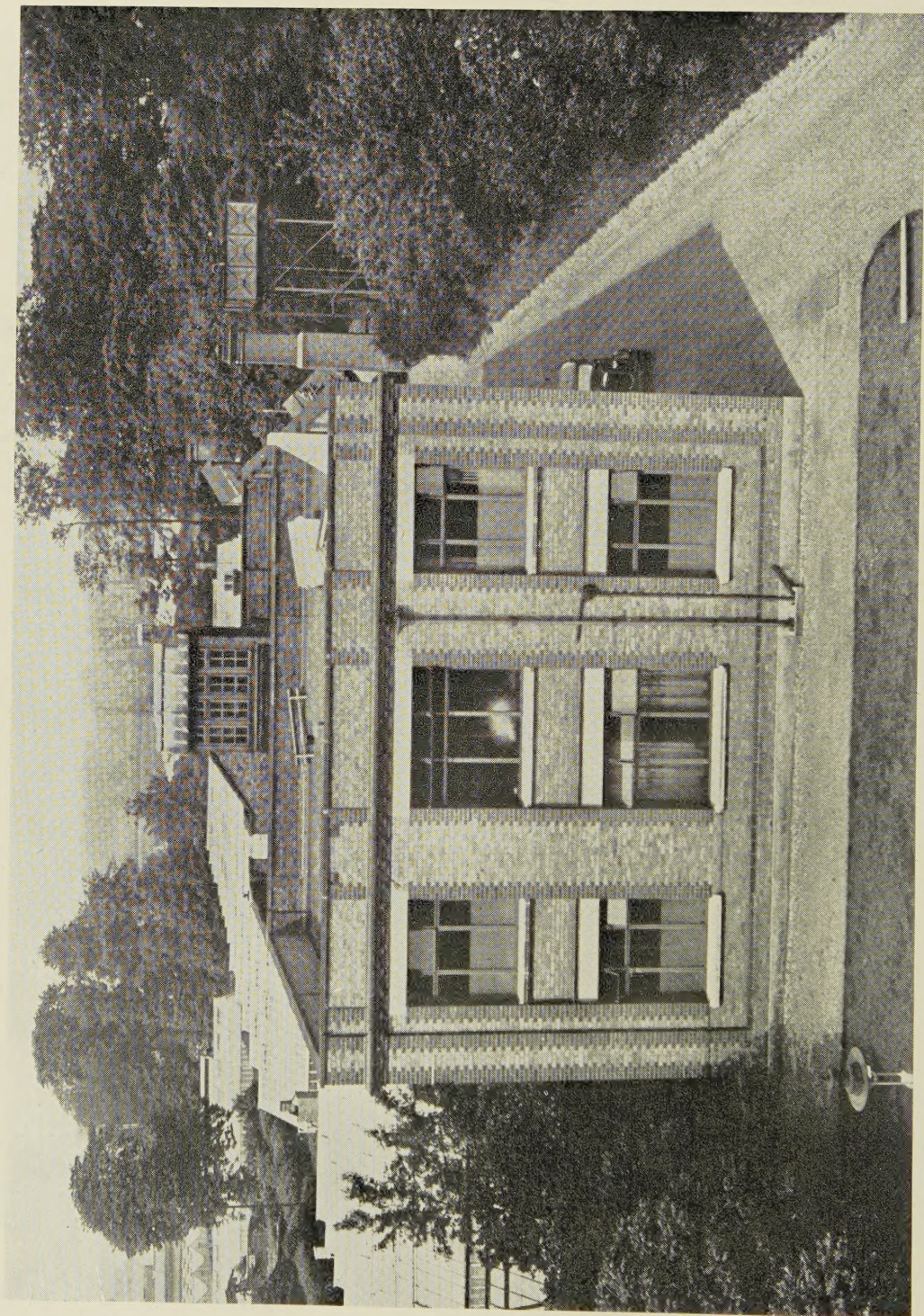
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INTRODUCTION

The work of the Research Station continues to make steady progress, despite the congested state of the laboratories and the leaky condition of the experimental glasshouses, resulting from enemy action during the war period.

Current Investigations

The successful use of alginic acid and certain alginates in the propagation of tomatoes was followed by larger scale experiments with tomatoes in the soil of the glasshouses. The yields obtained by applying both sodium alginate and alginic acid to soil without stable manure were rather higher than those from the control plots, and while they must be confirmed by further investigation, the results are promising. Milled seaweed was without effect, but this may have been due to the fact that it was applied in the dry state and remained dry in the soil despite the usual watering. Seaweed obtained from the South Coast had a depressing effect upon early growth. The continued use of leather waste in plots 4 and 7 of house 4 appears to be gradually improving the crop. This experiment is being continued.

The magnesium experiments in house 8 have been continued, but in the absence of any serious magnesium deficiency symptoms in the control plots, it was impossible to set any value on the results.

The experiment started in 1947 to determine the effect of omitting lime and phosphate from the tomato fertiliser treatment was continued. The yields from all plots were high, but there was a suggestion that in this, the third year of the experiment, the omission of both lime and phosphate may have commenced to reduce the yield.

In the variety trials very high yields were obtained from most varieties. Corleys, Downes Seedling, Bruinsma and Auchincruive 1a and 5, were amongst the heaviest croppers.

The cucumber crop was below average due to the presence of the root eelworm, *Heterodera marioni*, and also an invasion of bees which "set" the fruit in the summer. The insertion of a central core of either gravel or clinker in the beds resulted in a heavier crop. Once more, composted straw proved suitable as a substitute for horse manure in the preparation of cucumber beds.

In the mushroom experiments the addition of calcium alginate to the casing soil did not affect crop yield, but there was a suggestion that the addition of either sodium alginate or alginic acid to the casing soil was beneficial.

In the carnation experiments the plants grown in the ground and on a concrete bench produced a greater number of flowers per plant, than those in beds over either drain tiles or bricks. There was little difference between the first two methods of cultivation. In view of the fact that the plants were severely injured by frost early in both 1947 and 1948, these results may not be reliable and need confirmation.

The investigation of *Didymella lycopersici* has been continued. When cultures of this fungus were mixed with both steamed and unsteamed soil in open troughs, the fungus was found to persist in the steamed soil for eight weeks and in the unsteamed soil for seven weeks. Grown in soil in flasks, where it was free from the competition of other organisms, it survived for the duration of the experiment, a period of 21 weeks.

Growth in soil appears to be most active at a moisture content between 30 and 50 per cent. saturation. Aeration and the presence of organic materials, such as straw and partially decomposed stable manure, increase the amount of growth.

The addition of certain vitamins to an agar medium did not induce the production of either pycnidia or perithecia of *Didymella*.

Certain American varieties of tomato have been tested for resistance to *Verticillium albo-atrum*. Only one variety, Essar, remained free from infection. None of the varieties produced fruit suitable for the British market.

At regular intervals through the season, fungi have been isolated from the roots of tomato plants growing under normal commercial conditions in soil steamed for 1947. One fungus, as yet unidentified, appeared consistently associated with diseased roots from March to October. *Colletotrichum altrammentarium* was not isolated from the roots until June, but afterwards it was the commonest fungus isolated.

Samples of soil taken from immediately around the roots showed little correlation between the fungi isolated from the roots and those found in the soil.

Preliminary inoculation experiments with several of the fungi isolated, suggest that on very young tomato seedlings no definite lesions are produced.

A good deal of evidence has been obtained to show the ease with which virus diseases can be conveyed to tomato crops from tobacco, which is regarded as a constant source of infection in the tomato industry.

Under laboratory conditions 1 per cent. tannic acid in water when used as a spray was shown to be effective in reducing the spread of tomato aucuba mosaic, and there was little damage to the plant. The possibility of harm to human beings is a matter to be settled.

Tomato seeds infected with mosaic virus was treated with 400,000 röntgens of X-rays, without injury to the seed itself: all the seedlings grew normally but there was no destruction of the virus.

The investigations of the production of an antibiotic substance by *Penicillium patulum* in completely and also partially sterilised soil were completed.

Attempts were made to extract an antibiotic by both cold and hot water from non-sterile soil inoculated with *P. patulum* but without success. Other extraction methods are under investigation. Experiments with *Aspergillus terreus* were continued. This fungus produces several antibiotic substances including patulin on synthetic media. Preliminary results were confirmed and showed that *A. terreus*, like *P. patulum*, forms an inhibitory substance when cultured in sterile soil enriched with various sources of carbohydrate. It is suggested that this substance is not identical with the antibiotic formed by *P. patulum* because it is not active against the gram negative test bacterium *B. carotovorum*.

Investigation of the Tomato Leaf-miner (*Liriomyza solani* Her.) has been continued.

The feeding, mating, and egg-laying habits of the fly have been observed in detail: the period of incubation of the egg and duration of the larval life have been determined at recorded temperatures.

The average duration of the pupal instar in puparia formed during February was 57 days, and in those formed from April to June only 19 days, irrespective of prevailing temperatures.

The adult flies were not attracted to various baits employed in traps for other Dipterous insects. It has been concluded that infestation of young plants during the period of propagation arises solely from flies which emerge from puparia in the soil, derived from one or more generations of the previous year, and that timely steam sterilisation of the soil, in glasshouses or blocks of houses in which plants are to be raised, is essential in order to prevent infestation of seedlings or pot plants on nurseries upon which infestation might be deemed likely to occur.

Pyrethrum powder incapacitated flies which settled upon foliage to which it had been applied with much greater rapidity than powders containing either BHC or DDT: though flies, which were removed from the dusted leaf-surface shortly after they had become paralysed, recovered; it is highly probable that the deposit of powder, even after the loss of its lethal properties through exposure to light and a moist atmosphere, would make it difficult for the flies to insert their eggs into the dusted foliage, and thus minimise the severity of, but not entirely prevent, infestation.

Pyrethrum can be applied with safety to seedlings, whereas injury has been caused to them by application of powders containing DDT or BHC.

Some dead, but no living larvæ, were found in foliage to which a spray containing about 0.01 per cent. γ -isomer of BHC (i.e., a 32 per cent. emulsion of commercial BHC at a dilution of one part in 400 parts water) had been applied. Young pot plants were not injured by this spray.

A powder containing BHC killed the larvæ in their mines when applied to rather older pot plants, which, however, were injured beyond recovery by the dust. Only larvæ in an early stage of development were killed in their mines when the foliage of young pot plants was immersed in a 0.1 per cent. nicotine solution.

Young pot plants in which up to 20 larvæ were allowed to complete their development over a period of about 10 days suffered no more than a temporary check to their growth and made good plants after the larvæ had pupated. Full fed larvæ dropped on to soil the surface of which had been dusted with powder containing either DDT or BHC buried themselves in the soil and formed puparia normally: after emergence from the puparia, the flies succumbed on coming to the soil surface to which the powder had been applied a few days previously.

Work upon the British representatives of *Thysanoptera* has been continued. An investigation of the family Aeolothripidæ and of the glasshouse forms at present referred to the Sub-order Heliothripinæ has been completed.

By kind permission of the British Museum (Natural History) the specimens of Aeolothrips dating back to about the year 1836 have been mounted for microscopic examination, after which they were easily determinable as belonging to the species *Ae. tenuicornis* Bagn. with one specimen of *Ae. fasciatus* L.

Additional substances structurally related to azobenzene, i.e. with two linked phenyl or substituted phenyl groups, have been examined in the laboratory for ovicidal action on the eggs of the glasshouse red-spider mite (*Tetranychus telarius* L.). Many of the substances employed in these experiments have been synthesised in the laboratory.

No definite relationship between chemical constitution and ovicidal properties in respect to red spider eggs is evident as yet.

Suspensions of *bis* (*p*-chlorophenyl) methyl carbinol were found to have a high ovicidal action, but unsatisfactory control both of eggs and active stages of the mite was obtained in preliminary experiments in which this substance was dispersed as a "smoke." The proprietary product "Dimite" which contains 25 per cent. of *bis* (*p*-chlorophenyl) methyl carbinol was very effective against all stages of the mite when used as a wet spray. Cucumbers appear susceptible to injury by this compound.

Two organic phosphorus compounds *bis* (bis-dimethylamino) phosphonous anhydride and *bis* dimethylamino fluorophosphine oxide, which are termed systemic insecticides on account of their ability to become absorbed by plants and translocated throughout the plant, were used in experiments on the control of aphids on carnations by watering the plants with solutions of the compounds. The fluorophosphine oxide is much too injurious to plants to be employed in this manner, but the first compound, although a less powerful insecticide when used in this manner, was applied to the soil in amounts sufficient to give control of aphids within 16 days without injuring pot plants. A point of interest discovered in connection with these compounds was their pronounced acaricidal action when applied to the soil since red-spider mites were destroyed, more readily than aphids (*Myzus persicæ*).

Nitrification studies have been extended to include the following amino-acids: glycine, alanine, aspartic acid, glutamic acid, arginine, tyrosine and cystine. Ammonia formation has been investigated in addition to nitrification. With the exception of cystine all these amino-acids yielded maximum ammonia concentration within two to three days, after which the values fell as nitrification proceeded. In the case of cystine, ammonia formation was somewhat slower and this amino acid itself or its decomposition products exerted marked inhibiting effects on nitrification. Maximum ammonia and nitrate production were inversely related to the carbon/nitrogen ratio in those amino acids in which all the nitrogen is in the α amino position.

In general, the presence of alginate in addition to a source of nitrogen reduced nitrate production.

Results already reported on the effects of different levels of application of nitrogen on nitrification have been confirmed and, in addition, their effects on ammonia production have been examined.

Investigation of some of the chemical effects of steam sterilization have been continued. Evidence has been obtained which suggests that in soil which has been steamed more than once the effects of the second or subsequent sterilizations are not so pronounced as those which follow the first. Deliberate contamination of steamed soil with its unsterilized counterpart resulted in a lower maximum production of ammonia.

The main conclusion to be drawn from the investigations on mineral deficiencies in chrysanthemums is that varieties show wide differences in susceptibility.

Confirmation has been obtained of the striking effects which boron omission produces in chrysanthemum blooms. Soon after the buds open the outer ray florets show some distortion. They tend to assume a tubular shape and almost at the same time they become slightly twisted. Later the petals become incurved in varieties which are normally reflexed.

The last notes on plant physiological experiments appeared in the Annual Report for 1940. Work has now been resumed in the new department, but suffers from delay in obtaining equipment.

In collaboration with the Agricultural Branch of the Meteorological Office observations have been made of the frost protection afforded by a wood frame covered with standard Dutch lights. Minimal and maximal air temperatures and soil temperatures at different depths are being taken and the data is being analysed.

A preliminary survey of the distribution of tomato roots in a glasshouse border at the end of the crop has been made, using the soil-block technique.

Certain physiological diseases of tomato fruits are being studied from the physiological aspect.

Injection of a dye into the pedicel of tomato fruits by the wick-feed method has been tried as a means of following the transpiration stream. The method will require considerable improvement to be useful on a quantitative basis, e.g. in blotchy ripening investigations.

A preliminary attempt was made to determine if there are pronounced differences in the stage of development of the abscission layer at the "knuckle" of tomato fruits of varieties with different degrees of susceptibility to blotchy ripening, by finding the weight required to detach the fruits from the pedicel.

It has not been possible to start investigations on the soil atmosphere due to delay in delivery of apparatus. Trials with a soil tensiometer showed that the standard gauge-model has too great a lag in response to the changing soil moisture content in a glasshouse. Other forms will be tried when the equipment can be obtained.

The inhibition of chrysanthemum flower bud initiation is being studied, but owing to the exceptionally early season only one variety of chrysanthemum was available at the autumnal equinox without the full complement of flower buds having already been initiated. A time switch will be required for experiments on the effect of the interruption of the dark period to be undertaken earlier than this.



I. EXPERIMENTAL RESULTS OF 1949

I. TOMATOES.

The practice of grading the tomato fruits from the experimental plots, adopted in 1921, was continued:—

- A. "Pinks" number 5 to 7 and "Pink and White" 8 to 10 to the lb. These are the best quality of fruit in size, shape and colour; "Pinks" being slightly larger than "Pink and Whites."
- B. "Whites". These fruits are of good colour but smaller than "Pink and White."
- C. "Blues." This grade consists of fruits of good colour but larger and more irregular in shape than Grade A.
- D. "Roughs." Small mis-shapen fruits about the size of cherries.
- E. Blotchy fruits which ripen irregularly.
- F. Fruits showing Blossom End Rot.
- G. Fruits showing the lesions of Streak Disease.
- H. Fruits showing "Green Back."

(a) Houses 3 and 10; The use of alginates as a source of organic carbon in glasshouse soils

The successful use of alginic acid and alginates as a source of organic carbon in propagating soils has led this year to larger scale trials with the actual soil in the glasshouses, houses 3, 5 and 10 being used for this purpose.

In house 3, steam sterilized during the winter, two blocks of four plots each were arranged, to compare the effects of adding calcium alginate (1 oz. per square yard), sodium alginate (1 oz. per square yard), and milled seaweed (4 ozs. per square yard) with a control plot. Stable manure was not applied to the soil in any part of the house. Only in the case of sodium alginate was a greater yield recorded; the increase being nearly 14 per cent.

In house 5, seaweed from the South Coast of England, which had been allowed to lie in a heap for several months, was applied at the rate of 14 lbs. per square yard. The control plots having been steamed in the winter, received no stable manure. A very definite retarding effect was noticed during the first six weeks after planting, but the total crop was practically the same in both treatments.

In house 10 the experiment was a duplicate of that in house 3, except that the soil was treated with formaldehyde instead of steam and stable manure was applied to the control plots. The yields from the milled seaweed plots are low in comparison to those from the control plots, receiving horse manure. The calcium alginate plots produced a crop slightly less than the controls but the yield from the sodium alginate plots was slightly higher.

The effect of the milled seaweed is unexpected in view of the use which is made of this material in some areas, and it may be that having been added in a dry condition it did not become really wet during the season, for it could be seen easily when the soil was dug over at the end of the year.

The yields obtained from the sodium alginate plots need confirmation, but so far as they go they suggest that it may be used to replace horse manure, once we learn how to employ it to the best advantage. The results are given in Table I.

(b) House 4

The deficiency experiments in this house have been in progress since 1916. Once more the omission of either nitrogen or potash reduced the yield considerably and increased the amount of blotchy ripening. The omission of phosphate after all these years, however, hardly affected either the yield or the degree of blotchy ripening.

The continued application of leather waste to plots 4 and 7, has gradually increased the yield. This year it averaged 57.23 tons per acre, and the general appearance of the plants was excellent. The results are shown in Table II.

(c) House 7

In this house a new soil warming device was installed and tested, but unfortunately it broke down and increased temperatures were not obtained. It will be repeated in 1950.

TABLE II
VARIETY.—PLOTS 1-4 AND 7-10, DOWNE'S SEEDLING; PLOTS 5 AND 6, DUFFERN'S SEEDLING.
SOIL STEAM STERILIZED.

House	Plot	Treatment	Crop Weight in Lbs. per Plant					Total Tons per Acre	% Blotchy Ripening
			June	July	Aug.	Sept.	Total		
4	1	Complete artificials without nitrogen	1.88	1.28	1.06	1.04	5.26	39.97	—
	2	Unmanured	1.40	0.44	0.66	0.75	3.25	24.70	13.85
	3	Complete artificials	1.77	0.71	0.80	0.95	4.23	32.14	0.68
	6	Control	1.51	2.22	1.07	1.20	6.00	45.60	0.33
	4	Leather waste	2.49	2.32	1.16	1.74	7.71	58.59	1.53
	7	"	2.52	2.29	1.16	1.38	7.35	55.86	1.90
	8	Complete artificials plus dung	1.93	1.46	0.59	0.51	4.49	34.12	0.64
	9	" without phosphate	2.43	0.83	0.52	0.51	4.29	32.60	0.70
	10	" potash	1.32	0.64	0.10	0.14	2.40	18.24	37.06

(d) House 8

Two treatments calculated to correct magnesium deficiency were tested in this house. Two plots were untreated controls. Four plots were given three top-dressings of magnesium sulphate each at the rate of 2 ozs. per square yard, and in two plots the plants were sprayed four times with magnesium sulphate solution. Unfortunately, there was little magnesium deficiency on the control plots and so it was not possible to record the degree of improvement, if any, resulting from the treatments. The variety grown was Single Cross, but it showed up very badly as it was the sixth generation from the cross and had obviously broken down. The relatively low yields where the plants were sprayed with magnesium sulphate solutions, therefore, cannot be taken as reliable. It is clear that growers using the variety Single Cross, should obtain new seed from a first cross each year.

The results are set out in Table III.

(e) Houses 11 and 12; The effect of omitting lime, and phosphate from the fertiliser treatment

The experiments started in 1947 have been repeated.

Plots 1 and 2 in house 11 received standard treatment, while plots 3 and 4 received the same treatment without lime. Plots 1 and 2 in house 12 received standard treatment without phosphate while in plots 3 and 4 both lime and phosphate were omitted.

The results are given in Table IV. In this the third year of the experiment, the omission of both lime and phosphate seem to have reduced the yield, but it is still too early to attempt an explanation.

(f) Variety Trials

The variety trials in the Rose House were continued, the soil having been steam sterilized during the winter. The results are given in Table V. The yields obtained this year are so high that they were checked several times, to ensure their accuracy. It can only be assumed that the considerable amount of sunshine, and steam sterilization of the soil, are responsible. The house is, of course, an isolated one with no shade around it. Old mushroom compost at the rate of 30 tons per acre was applied to the surface of the soil before steaming and was steamed in with the soil. The full dressing of base fertilizer also was applied after steaming.

Reference must be made to some of the varieties.

Corleys, originally given to us by Mrs. Underwood, is a sunrise type, with medium to large fruit, fleshy and firm. It colours well.

Downes Seedling and Bruinsma have both maintained their past excellence and it is possible that Bruinsma may ultimately replace Potentate in the industry. Auchincruive 1A and 5A were raised at the West of Scotland Agricultural College. Both cropped well in these trials, yielding heavy crops of good quality fruit. They were relatively free from attack by *C. fulvum*, but conditions generally did not favour Leaf Mould, and it would be unwise to base an opinion of resistance to this disease on the experiences of 1949.

2. CUCUMBERS

The cucumber crop of 1949, although a little better than 1948, was below the average. This was due to the presence of eelworm in the borders and to invasions of bees during the summer months. For 1950 either chloropicrin or D.D. has been used after steaming in an attempt to eradicate the eelworm and so, improve the crop.

The experiments in 1949 included the provision of a core of either gravel or washed clinkers to serve as drainage inside the bed itself. The core was approximately triangular in section and occupied about half the mass of the original bed, which was about 18 ins. wide and 16 ins. deep. The remainder of the bed was composed of a mixture of two parts loam and one part strawy horse manure. In each case there was a definite increase in crop as a result of inserting the drainage core in the centre of the beds.

The other experiments were devised to compare composted straw, and composted straw plus stable manure in two different proportions with standard beds, adding the same proportion of loam as in the control beds. There was little difference in the weight of crop from the different beds, but the variation between crops receiving similar treatment, resulting from eelworm attack, prevents any definite conclusions from being formed. The work, however, confirms previous results which suggested that composted straw was as suitable as horse manure for cucumber work. The results are given in Table VI.

TABLE III
VARIETY.—HOUSE 7, E.S.5; HOUSE 8, SINGLE CROSS; HOUSE 9, POTENTATE.

House	Plot	Treatment	Crop Weight in Lbs. per Plant						Total Tons per Acre	Average Tons per Acre	% Blotchy Ripening
			May	June	July	Aug.	Sept.	Oct.			
7	1	Standard treatment ...	0.13	2.47	1.83	1.17	0.52	0.24	6.36	52.64	0.31
	2	" ...	0.18	2.50	2.07	1.44	0.57	0.21	6.97		—
	3	" ...	0.15	2.64	1.93	1.65	0.61	0.47	7.45		0.27
	6	Electric soil-warming with a new device ...	0.14	2.67	1.71	1.96	0.49	0.21	7.18		0.98
8	7	" ...	0.12	2.70	1.74	1.36	0.43	0.20	6.55	52.51	0.30
	8	" ...	0.06	2.43	2.00	1.68	0.58	0.25	7.00		—
	4	Control ...	0.02	2.31	1.38	1.05	0.59	0.90	6.25		0.64
	7	" ...	1.80	2.06	0.88	0.83	0.41	—	5.98		2.00
	2	Magnesium sulphate: Three top-dressings each of 2 ozs. per sq. yd.	0.08	2.27	1.51	0.95	0.80	0.28	5.89	46.76	0.34
	3	" ...	0.08	2.20	1.51	1.04	0.83	0.27	5.93		1.01
	5	" ...	—	2.19	2.07	0.27	0.78	0.28	6.59		0.91
	8	" ...	—	1.94	1.96	0.86	0.84	0.60	6.20		1.94
9	1	Sprayed four times with magnesium sulphate solution ...	0.09	2.05	1.58	0.98	0.55	0.16	5.41	43.20	0.37
	6	" ...	—	2.08	1.74	0.87	0.91	0.36	5.96		1.67
	1	Standard treatment, but nitrogen given as sulphate of ammonia ...	—	2.49	2.03	1.15	1.12	0.47	7.26	55.82	3.45
	2	" ...	—	2.85	1.90	1.07	1.06	0.55	7.43		3.63
3	3	Standard treatment ...	—	1.79	2.53	0.99	1.21	0.72	7.24	55.29	4.14
	4	" ...	—	1.89	2.37	0.97	1.32	0.76	7.31		4.10

TABLE IV
 VARIETY.—HOUSE 11: PLOT 2 AND 3, L.M.R. No. 1; PLOT 1 AND 4, E.S.1.
 HOUSE 12: PLOT 2 AND 3, L.M.R. No. 1; PLOT 1 AND 4, E.S.1.

TOMATOES

House	Plot	Treatment	Crop Yield in Lbs. per Plant					Total Tons per Acre	Average Tons per Acre	% Blotchy Ripening
			June	July	Aug.	Sept.	Oct.			
11	1	Standard	0.40	3.04	2.32	1.54	0.91	8.21	62.39	4.48
11	2	"	0.14	1.83	2.38	1.47	1.17	6.99	53.12	2.00
11	3	No lime	0.14	0.97	2.30	1.95	0.90	6.26	47.57	0.16
11	4	"	0.28	2.63	2.12	1.34	0.38	6.75	51.30	6.81
12	1	No phosphates	0.81	2.53	2.15	1.20	0.26	6.95	52.82	1.58
12	2	"	0.57	2.25	1.84	1.11	0.58	6.35	48.26	1.89
12	3	No phosphates and no lime	0.48	2.11	2.46	1.05	0.39	6.09	46.28	2.13
12	4	"	0.92	2.80	2.40	1.16	0.31	7.59	57.68	6.97

TABLE V
 VARIETY TRIALS. SOIL STEAM STERILIZED

TOMATOES

Plot	Variety	Crop Weight in Lbs. per Plant							Total Tons per Acre	% Blotchy Ripening
		May	June	July	Aug.	Sept.	Oct.	Total		
1	Corleys	0.06	3.57	4.18	1.80	1.67	0.56	11.84	89.98	2.96
6	Downes Seedling	0.01	3.65	3.74	1.97	1.01	0.73	11.11	84.43	6.93
7	Auchincruive 1A	0.08	2.73	3.96	2.12	1.48	0.54	10.91	82.91	5.41
5	Bruisna	—	2.98	3.22	1.91	1.34	0.48	9.93	75.46	5.24
8	Auchincruive 5A	0.03	2.51	3.92	1.89	1.10	0.31	9.76	74.17	4.40
12	Gerrards Ailsa Craig	—	1.83	4.04	2.08	1.39	0.32	9.66	73.41	3.62
3	L.M.R. No. 1	—	2.20	2.78	1.95	1.57	0.99	9.49	72.12	1.79
2	Stonors All Clear	0.02	2.77	3.25	1.48	1.34	0.46	9.32	70.83	6.97
11	P x P.	—	2.80	3.44	1.73	1.23	—	9.20	69.92	22.40
14	Scottish Chieftain	—	2.37	2.71	2.05	1.49	0.25	8.87	67.41	1.92
4b	362/48	—	1.27	3.75	2.68	1.10	0.13	8.93	67.86	2.01
4a	Brookfield	—	2.67	2.83	1.39	1.13	0.49	8.51	64.67	—
9	Freedom	—	3.21	2.58	1.36	8.97	0.24	8.46	64.29	.24
10	Culbin's Seedling	0.10	2.26	2.67	2.07	1.19	—	8.19	62.24	2.32
13	P x P (No. 3 plant)	—	2.05	2.45	1.44	1.22	0.22	7.36	52.93	1.76
	Perspex House	—	—	—	0.16	5.19	1.01	6.20	47.12	—
	Plastic	—	—	—	0.07	2.49	0.79	3.44	26.14	—
	Windolite	—	—	—	—	2.51	0.68	3.26	24.77	—

TABLE VI
VARIETY.—BUTCHER'S DISEASE RESISTER

CUCUMBERS	Treatment		Crop Yield in Lbs. per Plant							Total Tons per Acre	Average Tons per Acre	Total Flats per ft.	Average Flats per ft.
	Base	Beds	Mar.	Apr.	May	June	July	Aug.	Sept.	Total			
D2	Steamed	Coarse gravel core inside standard beds ...	2.17 1.12	11.48 12.11	8.59 8.37	9.47 10.27	2.06 2.33	12.25 10.28	1.99 2.86	48.01 47.44	72.01 71.16	1.50 1.49	1.49
E3	"	"											
D4	"	"											
F5	"	Clinker core inside standard beds ...	1.75 1.90	12.19 10.80	7.95 8.76	9.65 10.03	2.26 2.64	12.27 11.07	2.55 1.88	48.62 47.08	72.93 70.62	1.52 1.48	1.50
D3	"	Control: 2 parts loam, 1 part horse manure ...	1.00 2.33	12.52 10.93	9.78 7.47	9.67 7.62	2.13 1.91	12.17 8.78	2.03 1.25	49.30 40.29	73.95 60.43	1.54 1.26	
D5	"	"	2.19	10.51	7.89	8.52	1.44	9.07	1.29	40.91	61.36	1.28	1.35
E2	"	"											
E4	"	"	0.89	10.61	8.01	9.35	2.81	9.83	1.67	43.17	64.75	1.35	
F3	"	Straw composted 1 part, loam											
G4	"	2 parts ...	0.40	8.32	6.95	6.47	2.44	6.80	1.53	32.91	49.36	1.03	1.34
F4	"	"	—	13.59	10.61	7.17	5.43	14.01	2.30	53.11	79.66	1.66	
G5	"	Straw composted 3 parts, stable manure 1 part, loam 8 parts	0.53	11.33	9.69	9.82	2.37	7.19	2.41	43.34	65.01	1.36	1.29
F5	"	"	—	12.04	9.69	6.42	3.88	6.76	0.24	39.03	58.54	1.22	
G3	"	Stable manure 1 part, loam 2 parts ...	1.14	11.80	10.21	8.37	1.75	4.44	1.08	38.79	58.18	1.21	1.25
F2	"	"	0.35	10.67	7.87	5.96	3.88	11.73	1.24	41.70	62.55	1.30	
G2	"	Straw composted 1 part, stable manure 1 part, loam 4 parts	1.29	11.20	7.86	7.02	1.96	7.07	1.10	37.50	56.25	1.17	1.11
	"	"	0.05	12.51	8.49	4.49	2.63	5.20	0.16	35.53	50.29	1.05	

TABLE VII

MUSHROOMS

Bed	Compost	Casing Soil	Crop Weight in Lbs. per Sq. Ft.					Average Tons per Acre
			Feb.	Mar.	Apr.	May	June	Total
A4	Control: stable manure	...	—	0.61	0.61	0.38	0.06	1.66
B2	"	Soil and lime ...	1.10	0.48	0.21	0.19	0.01	1.99
C4	"	" " " "	0.86	0.39	0.35	0.36	0.06	2.02
D1	"	" " " "	0.94	0.39	0.29	0.13	—	1.75
E2	"	" " " "	1.04	0.30	0.21	0.14	—	1.69
F4	"	" " " "	0.83	0.22	0.30	0.24	0.06	1.65
B1	Manure and cotton seed meal	...	0.98	0.39	0.25	0.19	0.01	1.99
E1	"	" " " "	0.88	0.32	0.22	0.15	—	1.57
D2	"	Minor elements added	1.13	0.19	0.28	0.15	—	1.75
D4	Stable manure	"	0.93	0.42	0.38	0.16	0.02	1.91
C2	"	Sodium alginate added	1.06	0.34	0.29	0.28	0.06	2.03
D3	"	Calcium alginate added	0.57	0.53	0.25	0.19	—	1.54
F2	"	Alginic acid added	1.03	0.36	0.26	0.19	0.06	1.90
A1	Chopped straw treated with Adco	Soil and lime ...	—	0.41	0.37	0.27	0.08	1.13

TABLE VIII
BED NO. 1.—BEDS OVER A BRICK BASE. PLANTED JUNE 9TH, 1947.

Variety	No. of Plants	Flowers per Month												No. of Flowers per Plant	
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		Total
King Cardinal	66	10	40	24	80	23	152	224	165	95	75	42	30	960	14.54
Wivelsfield White	42	22	58	26	51	10	177	308	130	71	46	25	14	938	22.33
Pantaloon	30	7	20	12	16	3	93	139	52	19	14	8	—	383	12.77
Virginia	30	10	12	7	17	6	53	154	67	22	22	9	11	390	13.00
Mrs. Dixon	60	24	27	29	27	18	101	315	243	124	62	50	10	1,030	17.17
Spectrum Supreme	54	14	38	40	36	20	72	221	130	53	30	31	11	696	12.99
Maytime	6	1	12	5	16	4	49	30	34	26	8	5	—	190	31.67
White Maytime	36	11	33	34	51	12	140	127	104	73	52	24	8	665	18.47

TABLE IX
BED NO. 2.—BEDS IN RAISED CONCRETE BENCH. PLANTED AUGUST 16TH, 1947.

Variety	No. of Plants	Flowers per Month												Total	No. of Flowers per Plant
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		
King Cardinal	60	14	53	43	72	10	220	216	125	93	87	55	25	1,013	16.88
Wivelsfield White	36	28	101	17	32	14	162	298	122	77	53	34	11	949	26.36
Pantaloon	30	20	27	21	16	1	115	145	66	32	21	9	—	473	15.77
Virginia	42	25	18	20	46	16	125	161	96	68	50	25	9	659	15.69
Mrs. Dixon	48	22	49	56	60	43	137	286	246	148	96	61	16	1,230	25.62
Charming	24	3	9	9	8	13	73	86	37	23	7	14	3	295	12.29
White Maytime	18	17	41	18	45	14	110	100	88	48	36	17	—	534	29.67
Spectrum	18	7	22	17	24	9	43	96	47	20	18	16	10	329	18.27
Pink Treasure	12	1	5	11	14	5	64	51	27	24	29	18	8	257	21.42

TABLE X
BED NO. 3.—BEDS OVER A DRAIN TILE BASE. PLANTED JUNE 9TH, 1947.

Variety	No. of Plants	Flowers per Month												No. of Flowers per Plant	
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		Total
King Cardinal	60	10	19	19	58	30	125	192	135	76	45	41	6	756	12.60
Wivelsfield White	48	25	66	43	41	17	172	350	153	69	42	24	9	1,011	21.06
Pantaloon	48	13	40	20	42	9	90	181	79	33	21	7	—	535	11.14
Spectrum	48	13	33	63	33	17	61	211	147	56	31	19	10	694	14.45
Olivette	48	10	2	21	—	—	—	—	—	—	—	—	—	33	00.69
Charming	30	2	10	10	13	6	63	115	52	35	19	12	2	339	11.30
Clayton Crimson	30	3	3	10	27	15	113	89	36	45	25	14	5	385	12.83
Joy	12	—	4	8	9	1	31	27	12	7	4	2	—	105	8.75

TABLE XI
BED NO. 4.—BEDS OVER THE NATURAL GROUND. PLANTED JUNE 24TH, 1947

Variety	No. of Plants	Flowers per Month												No. of Flowers per Plant	
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		Total
King Cardinal	66	8	63	33	99	13	240	217	174	108	100	41	21	1,197	18.14
Wivelsfield White	42	45	144	18	41	10	193	448	161	80	52	42	10	1,344	32.00
Pantaloon	36	17	25	23	29	2	97	137	79	28	18	10	—	505	14.03
Olivette	48	10	9	20	—	—	—	—	—	—	—	—	—	39	00.81
Topsy ...	66	26	43	58	43	12	216	305	131	88	53	29	18	1,062	16.09
Pink Treasure	30	1	7	48	45	6	77	123	83	49	41	35	15	620	20.67
Spectrum Supreme	30	2	15	29	32	12	71	164	70	26	24	20	12	477	15.90

3. MUSHROOMS

Previous experience of the addition of cotton seed meal to horse manure during composting had given promising results, but nothing more could be done during the war years owing to lack of supplies. In 1948, however, a very small quantity was obtained for experimental purposes. Unfortunately, it arrived too late to be mixed with the manure during the first turn, and as the results show (Table VII) the addition at the time of the third turning failed to improve the compost.

The addition of a number of minor elements to the casing soil was also without effect, but there is some evidence to suggest that the crop is improved by the addition of sodium alginate and alginic acid. These trials, however, must be repeated before a definite conclusion can be reached.

The compost prepared by fermenting chopped straw with Adco accelerator produced only a small crop, although the quality of the mushrooms was good.

4. CARNATIONS

The experiments started in 1947 were continued, the results being given in Tables VIII to XI.

The results indicate little difference in numbers of flowers per plant between the carnations grown in the ground and in the concrete tank, both being definitely superior to those in beds over either drain tiles or bricks. It will be remembered that owing to failure of the old heating system these plants suffered very severe frost injury in both 1947 and 1948. In view of this and many other difficulties encountered during the two and a half years of this crop, however, it is doubtful if these results are reliable.



II. PLANT DISEASES

I. DIDYMELLA INVESTIGATIONS

By P. H. WILLIAMS

(A) Some factors influencing growth of the fungus in the soil

In the Thirty-fourth Annual Report for 1948, methods were described by which spores of *Didymella lycopersici* might be obtained readily in pure culture. This has enabled some pure culture studies to be made of certain factors likely to influence the growth and survival of *Didymella* in the soil.

The method employed was as follows. Steam sterilized maiden soil containing either stable manure or old cucumber bed was air-dried and sifted through a 3-m.m. sieve. Weighed quantities of the airdry soil were placed in flasks and sufficient distilled water added to bring it to a pre-determined moisture content. The flasks were then sterilized in the autoclave and inoculated by adding a measured quantity of a spore suspension taken from cultures on either fumigated tomato stems or agar containing tomato juice. The soil cultures were incubated on the laboratory bench. At intervals, the soil was removed from certain of the flasks, thoroughly mixed and 25g. samples plated on Dox agar containing rose bengal.

(a) Soil moisture

In a preliminary experiment, 90 g. of air-dried soil were weighed out into 100 ml. flasks. These were divided into five groups and distilled water was added to bring the moisture content to 100 per cent., 75 per cent., 50 per cent., 30 per cent., and 10 per cent. of saturation. The flasks were inoculated by adding 1 ml. of a spore suspension derived from a culture on fumigated tomato stem.

The first samples were plated three weeks after inoculation and subsequently every fortnight. In sampling one flask was taken from each series. The results are shown in Table I and show the total numbers of colonies in 10 plates poured from each flask.

TABLE I
THE EFFECT OF SOIL MOISTURE ON GROWTH OF DIDYMELLA

Time	Moisture Content of the Soil				
Weeks	100%	75%	50%	30%	10%
3	0	3	1	36	0
5	2	2	9	461	2
7	6	4	1	296	0
9	2	2	1	450	0
11	1	1	0	906	3
13	2	0	2	1,403	3
15	0	19	1,522	1,543	27
17	3	40	2,183	2,806	5
19	10	18	2,214	2,409	5
21	9	18	2,311	2,259	3

It will be seen that for the first 13 weeks there was decidedly better growth at 30 per cent. saturation. Subsequently three of the other series showed a sudden increase in numbers, those at 50 per cent. particularly so. The cause of this increase is unknown. Possibly it may have been due to the onset of higher temperatures in the laboratory as the increase occurred at the end of March.

A second experiment was carried out differing in two respects: (a) the soil was a mixture of maiden turf and old cucumber bed and (b) 300 g. of soil were used instead of 90 g. Growth was poor and sporadic and no definite conclusions could be drawn from the results obtained. It seems probable that the failure to grow might be due to several causes. In the first place, the soil might not contain a sufficient quantity of suitable organic matter. Secondly, the fungus may not grow readily in the interior of the soil mass but may be confined mainly to the surface layers. Since the ratio of surface to volume diminishes with increase in volume, it would be expected that if the fungus grows mainly near the surface of the soil, higher counts would be obtained when 90 g. were used than when 300 g. were used.

To test both these possibilities, a new batch of the same maiden soil was obtained to which stable manure was added. This was air-dried and sieved as before. Flasks were prepared from each soil, one series containing 90 g. and one series 300 g. Each series was subdivided into three sub-series, one of which was adjusted to 75 per cent. saturation, one to 50 per cent. and the third to 30 per cent. After sterilization, the 300 g. flasks received 2 mls. of spore suspension and the 90 g. 1 ml. These quantities were chosen in order to repeat the procedures used in the previous experiments. The results are shown in Table II over a period of seven weeks after inoculation.

It will be seen that the soil containing stable manure supported more growth than that containing the more completely decomposed old cucumber bed. Furthermore, the flasks containing 300 g. of soil gave smaller counts than those containing 90 g. It was observed that even in the flasks which showed growth on the surface of the soil, no visible growth was apparent in the interior. This suggests that the fungus does not penetrate very deeply into the soil, although obviously this will depend on the texture of the soil.

TABLE II
GROWTH OF *DIDYMELLA* IN TWO SOILS WITH DIFFERENT AMOUNTS OF ORGANIC MATTER

Time	300 g.						90 g.					
	Old Cucumber Bed			Stable Manure			Old Cucumber Bed			Stable Manure		
Weeks	30%	50%	75%	30%	50%	75%	30%	50%	75%	30%	50%	75%
3	1	3	0	174	290	0	60	182	184	299	249	87
5	3	39	0	237	245	1	75	170	170	390	413	138
7	3	59	23	285	200	0	73	241	241	315	438	147

So far as moisture content of the soil is concerned, it appears that *Didymella* will grow over a wide range.

(b) Organic matter

Following up the suggestion that organic matter such as stable manure or straw would favour the growth of *Didymella*, further experiments were carried out. Wheat chaff and short partly composted stable manure were air-dried and reduced to powder in a hammer mill in order to facilitate their incorporation in small quantities of soil. The soil used was the mixture containing old cucumber bed previously employed. The powdered materials were incorporated at rates of 1 per cent., 5 per cent. and 10 per cent. The mixtures were weighed out in 100 g. lots into 250 ml. flasks and the moisture content adjusted to 50 per cent. of saturation. After sterilization, the flasks were inoculated with 1 ml. of spore suspension. The results are shown in Table III and IV. As before the figures are the total, number of colonies developing in 10 plates.

TABLE III
THE ADDITION OF STRAW TO SOIL

Time	0%	1%	5%	10%
Weeks				
3	131	379	943	1,054
5	268	573	916	1,145
7	267	700	1,289	1,442
9	227	599	802	1,376
11	250	598	1,318	1,471

TABLE IV
THE ADDITION OF STABLE MANURE TO SOIL

Time	0%	1%	5%	10%
Weeks				
3	243	251	484	541
5	310	196	513	689
7	178	131	411	467
9	367	224	458	700
11	304	393	613	777

The addition of fresh straw markedly stimulated the growth of *Didymella*. Partly decomposed manure was not so effective. The results of these experiments suggest that the presence of straw either in the soil or as a mulch may favour the development of *Didymella*. Orth¹ has stated that the addition of fresh stable manure to the soil caused an increase of the disease compared with unmanured plots. The suggestion that straw may favour the growth of *Didymella* is also supported by the observation previously reported² that it grew and fruited on fumigated straw in test tubes.

(B) Growth on unsterilized soil

In the experiments so far described the fungus was free from the competition of other organisms, and was grown in small volumes of soil. To test its growth and survival under more natural conditions, 3 kilo lots of airdry soil were weighed out into glazed earthenware jars. Five jars were used in which the moisture contents were adjusted to 100 per cent., 70 per cent., 50 per cent., 30 per cent. and 10 per cent. of saturation. In the lowest concentration, the desired moisture content was obtained by adding the required volume of a spore suspension derived from infected stems. In the remainder the required volume of liquid was obtained by diluting this basic volume of spore suspension. Thus each jar received the same amount of inoculum. The pots were kept in the laboratory.

Samples of the soil were removed at fortnightly intervals. The contents of each jar was tipped out, well mixed and 300 g. samples taken. Part was used for planting out *Didymella*. The remainder was used to inoculate tomato plants, variety Potentate. The plants were inoculated when potting on into 6-in. pots. A space about 3 ins. in diameter and 1 in. deep was left around the collar of the plant and this was filled with the soil to be tested. Ten plants were inoculated with each sample and there were 10 controls. The plants were examined for infection after five weeks.

The fungus was recovered both in the plates and from the inoculated plants from all the series of soils except that kept at 10 per cent. saturation. Recovery was, however, rare, showing that the fungus did not spread uniformly throughout the soil mass. In view of the results reported above, it is possible that the poor growth was mainly due to lack of organic matter and the experiment will be repeated in the coming season.

(C) Survival in steamed and unsteamed soil

A quantity of soil containing stable manure was screened through a $\frac{1}{4}$ -in. mesh sieve. Part was then steamed for half an hour in the laboratory in a small steaming apparatus. Equal quantities of steamed and unsteamed soil were placed in glass tanks and sufficient water added to the unsteamed soil to bring its water content up to that of the steamed soil. After standing for 48 hours, equal quantities of a culture of *Didymella* on sand and oatmeal were mixed with the soil.

Samples for plating were withdrawn twice a week over a period of 114 days. It was found that during the first 80 days the number of colonies from the steamed soil was substantially greater than from the unsteamed. Thereafter the numbers were very small and there was no significant difference between the treatments. How far the differences were due to the favourable effect of steaming on the growth of *Didymella* is uncertain. Throughout the experiment the number of contaminants was usually considerably higher in the plates from the unsteamed soil. It seems probable therefore that the results obtained were due at least in part to competition in the plates. It is interesting to note the persistence of *Didymella* over so long a period. It is possible that this may be due to the presence of dark carbonised hyphæ in the cultures used for inoculation.

(D) The effect of Tomato Juice on growth and fruiting of *Didymella*

It was reported in the Thirty-fourth Annual Report that the addition of tomato juice stimulated the formation of pycnidia on an agar medium. This experiment has been repeated on several occasions.

The juice was extracted in a hand press and then filtered through paper pulp to remove debris and centrifuged to remove finer particles. It was then divided into three portions. One portion was filtered through a filter pad to remove colloids. One portion of this filtered juice was then sterilized by filtration and added aseptically to sterile Hawker's medium A which had been previously melted and cooled. The addition was made at the rate of 2 mls. juice to 15 mls. agar. A second portion was added to the medium at the same rate and sterilized by steaming for 30 minutes on three successive days. With a third portion the procedure was the same except that the tubes were autoclaved.

The second and third portions of the crude juice were steamed for 30 minutes and autoclaved for 20 minutes at 120° C. respectively. They were then filtered through Whatman No. 1 filter paper. The subsequent procedure was the same as with the untreated juice.

The responses to treatment were variable but with all treatments pycnidia were formed in some tubes. Untreated juice sterilized by filtration gave the best results. In all cases, pycnidia were most numerous on areas where the mycelium had collapsed but in some tubes they were fairly abundant under the mycelium. Only in one experiment did pycnidia develop in the control tubes. They were much less abundant than in those receiving tomato juice.

(E) The effect of Wheat Straw Extracts on growth and fruiting of *Didymella*

In 1948, it was found that *Didymella* would grow and fruit on wheat straw sterilized by fumigation with propylene oxide. An attempt was made to extract a possible active principal by soaking 100 g. of wheat chaff in 1 litre of water for 48 hours and also by autoclaving a similar mixture. The cold water extract was treated in the same way as the tomato extracts described above. The hot water extract was sterilized by filtration and added to the sterile medium. No influence on pycnidium formation was observed.

As it was thought that the extracts might have been too dilute, a more comprehensive experiment was made this year. Five lots of wheat chaff of 50 g. each were weighed out into beakers. Distilled water was added at the rate of 50, 100, 150, 200 and 250 mls. respectively and the mixtures were allowed to stand overnight. They were then steamed for 30 minutes and the liquid squeezed out in a small press. The extracts were sterilized by filtration and added to sterile Hawker's medium at the rate of 2 mls. to 15 mls. medium.

The addition of the extracts stimulated vegetative growth but not pycnidium formation. The factor which stimulated fruiting on the wheat straw would therefore appear to be different from that which occurs in tomato juice.

(F) The effect of certain vitamins on growth and fruiting of *Didymella*

Within recent years considerable attention has been paid to the role that certain vitamins play in the nutrition of fungi. It has been shown that growth and fruiting of some fungi is stimulated by the addition of such substances as aneurine (Vitamin B₁) and biotin (Vitamin B₆) to media. In view of the results achieved with tomato juice, it seemed possible that *Didymella* might be stimulated to fruit if provided with certain of these substances. Accordingly preliminary experiments have been carried out using aneurine, biotin, pyridoxine, p-aminobenzoic acid and inositol.

In these experiments aneurine and pyridoxine have been used at the rate of 0.5, 1.0, 2.5, and 5 µg. per 100 mls. (1 µg.—one millionth of a gram.) Biotin was used at 0.005, 0.01, 0.02, 0.04 µg. per 100 mls. and p-amino benzoic acid at 0.25, 0.5, 1.0, and 2.0 µg. per 100 mls. Inositol is required at comparatively high concentrations and was used at 5, 10, 20 and 50 mg. per 100 mls. The required concentrations were obtained by diluting solutions of convenient strength so that 1 ml. of the final dilution could be added to 15 mls. of Hawker's medium A. The substances were either added before sterilization or sterilized by filtration and added aseptically to the sterile medium. In either case, the cultures were grown on slopes in 6 in. by 1 in. tubes.

Of the substances tested, only biotin produced any effect on growth. With as little as 0.01 µg. per 100 mls. growth was slightly better than in the control and there was greater production of carbonised hyphæ. With 0.02 µg. the effect was more pronounced but with the highest concentration, growth did not quite cover the whole of the slope. No effect was observed on fruiting.

The experiments are being continued.

Thanks are due to Messrs. Vitamins Ltd., for supplying samples of aneurine, biotin and pyridoxine.

REFERENCES

- ¹ ORTH, H., *Kranke Pflanze* (1939) 16; 155.
- ² WILLIAMS, P. H., *Rep. Exp. Res. Sta., Cheshunt*, 1948, 24.

2. THE RESISTANCE OF SOME AMERICAN TOMATO VARIETIES TO VERTICILLIUM WILT.

By P. H. WILLIAMS

Seed of certain American tomato varieties was received this year. These varieties were Simi, Southland, Essar and Riverside and were said to be resistant to *Verticillium* Wilt.

The plants were propagated in the usual manner. Unfortunately germination was poor, particularly in Riverside and Essar. In the case of Riverside seed saved in 1945 from plants used in

former experiments was employed as a substitute. Potentate was grown as a susceptible control. When ready, the plants were potted into 10-in. pots and inoculated by placing a piece of a culture of *Verticillium albo-atrum* under the ball of roots. The plants were inoculated on March 22nd and 23rd.

The most recently isolated culture of *V. albo-atrum* in the Station collection was used for inoculating the plants. Unfortunately it had been in culture for several years and appeared to have lost some of its virulence. Hence the observed symptoms were very mild, being confined to yellowing and wilting of the older leaves. No differences were observed in this respect between Potentate and the other varieties.

At the end of July, the plants were cut out and observations made on the presence or absence of browning in the wood. Isolations were made from every plant which showed browning to confirm the presence of *Verticillium*. The results of these tests were as follows:—

Southland	...	...	...	6 plants infected out of 8
Simi	...	...	...	5 " " " 8
Essar	...	...	...	0 " " " 4
Riverside	...	...	...	8 " " " 8
Potentate	...	...	...	8 " " " 8

Of the varieties tested only Essar remained completely healthy, the remainder showing varying degrees of susceptibility.

None of these varieties would be suitable for culture in Great Britain, all of them except Riverside being of the large flat canning type. Riverside is more regular in shape but large and fleshy and would probably not be favoured in the market.

3. BROWN ROOT ROT OF THE TOMATO

By MARION EBBEN

During the whole season tomato plants growing under normal glasshouse conditions have been examined at regular intervals for symptoms of brown root rot. Isolations of fungi were made at the same time and it was attempted to correlate the nature and extent of the lesions observed with the fungi isolated. The roots of pot plants growing in the greenhouse have also been studied, and sections of roots of varying ages have been examined microscopically. Inoculation experiments have been carried out on seedling plants, using some of the fungi isolated from the tomato roots.

The plants used in this investigation were grown in a block at one end of a glasshouse and received the same treatment as the other plants in the house. The soil had been steamed in the autumn of 1947 and treated with formaldehyde in autumn, 1948. Before planting out in 1949 it received a dressing of stable manure and standard fertilisers. The young plants were first examined 11 days after planting out and thereafter at fortnightly intervals throughout the season, until the plants were cut out in October. Two plants were dug at the same time from similar positions in different rows, so that as far as possible the remaining plants were spaced evenly.

Microscopic examination of roots

The types of lesions observed on the roots throughout the year could be divided generally into five main types.

(1) Lesions caused by *Colletotrichum atramentarium* were characterised by the complete rotting away of the root cortex, leaving a brittle vascular cylinder on which sclerotia could generally be observed. These lesions were found on roots of all sizes, excepting the fine fibrous rootlets, and sometimes extended several centimetres along the root.

(2) Pale brown lesions of varying size were observed on small and larger roots. The lesions varied from very small round spots, which in some cases were probably due to the breaking off of lateral roots, to brown patches sometimes several millimetres in diameter on the large roots. These larger lesions occasionally girdled the roots, but apparently only the superficial tissues were affected.

(3) On some of the larger roots darker lesions in which the browned cortex appeared split and seemed to be flaking off, were noticed. These were probably a later stage in the development of the lesions in (2) above.

(4) The most severe lesions associated with brown root rot are corky swollen areas generally several centimetres in length and girdling the whole root. The increase of the root diameter in these areas seems to be due to the considerable increase in the cortical tissue which is dark brown in colour, furrowed and split. These lesions were only observed on the largest roots.

(5) On many of the plants examined the cortex at the base of the stem at soil level and just below was rotting. In the early stages the lesions appeared as brown dry patches on the stem, later the whole stem base was girdled with a black cortical rot, which often extended downwards to the upper parts of the main roots. Diseased tissue was almost entirely confined to the cortex, but in a few plants the xylem of the highest lateral roots was slightly affected.

The roots of the plants grown in 10-in. pots in the greenhouse showed fewer lesions and more fibrous root than those from the border. The lesions observed were of types (2) and (3) and no corky swellings or basal rotting developed. None of the roots of these potted plants, however, were as large as those on which corky swellings were observed, and from a comparison of root size and the types of lesion which develop it seems that the size of the root may be one of the factors which determines the type of lesion.

The types and numbers of lesions on the roots did not show any regular variation throughout the season, some of the roots examined late being as clean as those dug earlier. It was noticeable that when the roots were relatively clean the amount of fibrous root was greater, and it seems that infection leads first to the decay and disappearance of most of the fibrous root system. All the lesions examined were cortical rots, although the vascular cylinder was occasionally affected when the cortex had almost completely rotted away.

The roots examined in March and April were relatively clean, some of the smaller roots showed signs of decay and on larger roots there was some splitting and flaking off of the cortex. *Colletotrichum* symptoms were not definitely identified until the beginning of June. The swollen corky lesions (4) were not seen until the middle of July, but from then onwards all the plants examined showed some development of this type of lesion. Basal rot of the stem (5) was noticed in its early stages towards the end of June, and became more severe later in the season. When all the plants in the plot had been examined, it was found that 57 per cent. showed symptoms of this basal rot.

Examination of the roots indicated that the rotting of smaller roots is continually being counteracted by the development of new roots, which in turn may become rotted. The larger roots are rarely rotted completely away, but if severely affected can be of little value to the plant.

Microscopic examination of roots

On fresh material hyphae could sometimes be seen on the root surface and in the brown superficial cells of lesion tissue, but no extensive penetration of the root tissue was observed.

Microtome sections were prepared from roots dug at different times during the season, and from lesions of varying stages. Examination of these sections show that the lesion tissue consists of rather large irregular cortical cells with lignified walls, surrounding the normal cortical tissue. Some of the cell walls in this outer cortical layer seemed to be disintegrating, and the tissue was generally squashed by the pressure of the microtome knife. Fungal hyphae were generally lacking from the sections, but in some, small spores and swollen hyphae-like structures were seen in the disintegrating cortical tissue. In some roots the lignification of the cortical tissue extended locally inwards to reach the xylem but in most of the sections the vascular cylinder did not appear to be affected. The sections show that the root rot is primarily cortical, and only in the more extensive lesions does the cell disintegration reach the phloem and vascular cylinder.

Isolations of fungi from roots

Isolations of fungi were made from roots which had been well washed in running water and then surface sterilised for five to 10 minutes in a freshly prepared 7 per cent. solution of calcium hypochlorite. The isolations were made on Dox and potato glucose agar. At the beginning of the season when the roots were relatively clean, isolations were made from healthy roots as well as those with brown lesions. Later isolations were made from corky cortex and basal rot tissue. Fungal growth was obtained from approximately 70 per cent. of the root pieces tested, and nearly 500 isolations made.

The most frequently isolated fungi were:—

Colletotrichum atramentarium.

Fusarium spp.

an unidentified fungus with grey aerial mycelium and no spores which was labelled K.

Chaetomium cochliodes.

Other species isolated less frequently were:—

Volutella ciliata.

Petriella asymmetrica.

Myrothecium roridum.

Humicola sp.

Species of *Trichoderma*, *Phytophthora* and *Alternaria* were isolated very occasionally.

Table I shows the distribution of the most frequent species throughout the season, expressed as a percentage of the total number of fungi isolated each month.

TABLE I
PERCENTAGE DISTRIBUTION OF FUNGI THROUGHOUT THE SEASON

Fungus	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
<i>Colletotrichum</i> ...	—	—	5	20	42	23	49	50
K ...	19	9	28	26	25	23	20	15
<i>Fusaria</i> ...	16	24	18	42	10	30	13	15
<i>Chaetomium</i> ...	30	38	22	4	5	6	5	4
<i>Volutella</i> ...	3	9	9	—	14	13	5	8
<i>Petriella</i> ...	30	9	15	—	3	2	1	—

Although no strict comparison can be drawn between the numbers of different fungi isolated at succeeding stages during the season, since the degree of rotting of the roots did not show any regular variation, certain points of interest are brought out by comparing the frequency with which different species were isolated.

Colletotrichum atramentarium was not isolated until the end of May, but occurred far more frequently during June, and accounted for a high proportion of the isolations throughout the rest of the season. Isolations of *Colletotrichum* were 28 per cent. of the total number of isolations during the year.

Chaetomium cochliodes was isolated frequently during March, April and May, but the numbers dropped suddenly in June and only a few isolations of this fungus were made throughout the rest of the season. The drop in the numbers of *Chaetomium* isolates corresponds with the increase in the numbers of *Colletotrichum*, and it was also noticed that although no *Colletotrichum* was isolated from the roots of the pot plants, *Chaetomium* was frequent. In the laboratory the growth rate of *Chaetomium* on synthetic media and steamed soil was greater than that of *Colletotrichum*, and it may be that at the beginning of the season *Chaetomium* is able to spread rapidly through the soil and act as a weak pathogen on the roots, before the *Colletotrichum* becomes effective.

The *Fusaria* isolated were mostly microconidial forms and no attempt was made to identify them. The wide variation in the numbers of *Fusaria* isolations suggest that they were non-pathogenic forms, and no symptoms of *Fusarium* wilt disease were seen on the plants.

The grey non-sporulating fungus K accounted for a regular proportion of the isolations throughout the season, the largest number of isolations being made in May, after which the numbers decreased gradually. During the whole season 20 per cent. of the isolations were of this fungus and its regular association with the roots suggests that it may be partly responsible for the root rotting, either as a pathogen or secondary saprophyte.

Of the less frequently isolated fungi *Petriella asymmetrica* was relatively common during the first three months, but occurred only sporadically throughout the rest of the season. The numbers of isolations of *Volutella ciliata* increased during July and August, but were never very high. *Trichoderma* and *Phytophthora* were isolated occasionally throughout the season; *Phytophthora* generally from root lesions and *Trichoderma* from basal rot tissue. Three isolations of *Myrothecium roridum* were made during the year, one in April from a root lesion, and the other two at the beginning of October from a rotting stem base. A few isolations of *Humicola* were made in March, April and May from root lesions, later in the season this fungus was found in decaying stem cortex and corky root cortex.

The isolations made in the first three months of the season were from roots with brown lesions of types (2) and (3), and from apparently clean roots, later isolations were made from severe lesions and the numbers of the different species isolated from the various types of lesion are shown in Table II.

TABLE II
PERCENTAGE OF FUNGI ISOLATED FROM DIFFERENT TYPES OF LESION

Type of Lesion	Fungi Isolated	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
Brown Lesions	<i>Colletotrichum</i>	—	—	5	23	43	65	74	84
	K	20	5	28	29	25	5	4	—
	<i>Fusarium</i>	9	24	18	45	9	20	17	6
	<i>Chaetomium</i>	32	44	22	3	7	—	—	—
	<i>Volutella</i>	3	11	9	—	13	8	4	10
2 & 3	<i>Petriella</i>	29	11	15	—	2	—	—	—
Corky Roots	<i>Colletotrichum</i>					33	4	—	38
	K					33	40	66	33
	<i>Fusarium</i>					16	20	11	20
	<i>Chaetomium</i>					—	8	6	—
	<i>Volutella</i>					8	28	17	4
4	<i>Petriella</i>					8	—	—	—
Basal Rot.	<i>Colletotrichum</i>						16	66	44
	K						8	7	12
	<i>Fusarium</i>						33	13	20
	<i>Chaetomium</i>						16	10	12
	<i>Volutella</i>						16	—	12
5	<i>Petriella</i>							4	

The isolations of each fungus are expressed as a percentage of the total number of isolations made from each type of lesion. During the first three months practically all the isolations came from brown lesions, although a few isolations of *Chaetomium*, K, *Fusarium* and *Petriella* were made from roots which did not show any definite lesions. Occasional isolations were made from brown lesions at the stem base in June and July, but this type of lesion did not become severe until August when numerous isolations were made from this tissue.

Table II shows that of the large number of isolates of *Colletotrichum atramentarium* the greater proportion came from brown lesions and from basal rot tissue, isolations of this fungus from corky roots being very variable. The highest proportion of isolates of the fungus K came from the corky roots during the last four months of the season, whereas the number of isolations of K from brown lesions fell very suddenly in August, although this fungus accounted for quite a high proportion of the isolates from these lesions earlier in the season. *Chaetomium cochliodes* is shown to be chiefly associated with brown lesions during March, April and May, and with the basal rot tissue from August to October. *Fusarium* spp. and *Volutella ciliata* were found associated with all three types of lesion in varying numbers. *Petriella asymmetrica* was almost entirely confined to roots with brown lesions at the beginning of the season, and seems to be only rarely associated with the severer types of lesion.

Effect of isolated fungi on tomato seedlings

Several of the fungi isolated from diseased roots were used in reinoculation experiments, on young seedlings under controlled laboratory conditions. Two methods were employed. In the first, seeds were germinated in sterile Petri dishes, and the radicles inoculated when about 2 mm. long. Seven fungi were used as inoculants, and three methods of infection were tried. All the seeds used were surface sterilised and washed in sterile water before being germinated. The methods of inoculation were as follows:—

- A. Sterilized seeds were soaked in spore and mycelial suspensions of the seven inoculants, and then germinated.
- B. Drops of spore and mycelial suspensions were placed on young radicles.
- C. Blocks of agar culture were placed in contact with the radicles.

Soaking the seeds in a fungal suspension did not affect the germination. Seedlings on which lesions developed were removed from the dishes, surface sterilized and placed on agar slopes. Ten days after inoculation all the remaining seedlings were similarly treated. The percentage of radicles from which the fungal inoculant was reisolated is shown in Table III.

TABLE III
PERCENTAGE OF ROOTS FROM WHICH FUNGI WERE REISOLATED

Method of Inoculation	<i>Colletotrichum</i>	<i>Chaetomium</i>	K	<i>Petriella</i>	<i>Myrothecium</i>	<i>Volutella</i>	<i>Humicola</i>
A	17	13	10	10	—	—	7
B	7	80	7	57	37	7	—
C	13	10	—	30	13	26	3
Average	12	34	5.6	32	17	11	3

Localised brown lesions were seen on radicles inoculated with *Chaetomium*, *Myrothecium* and *Colletotrichum*, small brown flecks appeared on those treated with K and *Humicola*, while those inoculated with *Petriella* and *Volutella* showed no localised lesions, but a slight discolouration of the radicle. Brown lesions also developed on the hypocotyls of seedlings treated with *Myrothecium*. It was noticed that few reisolations were made from lesions which appeared within two or three days of inoculation and were immediately removed, and it is possible that the epidermal cells of the radicle were killed before penetration took place.

From the numbers of reisolations it appears that *Chaetomium* and *Petriella* were most effective in penetrating the radicles, but whereas inoculation with *Chaetomium* produced numerous small lesions, *Petriella* did not appear to produce any distinctly adverse effects.

Colletotrichum and K were not very effective. *Myrothecium roridum* produced numerous lesions both on the radicles and hypocotyls but was not always reisolated. Few reisolations were made from radicles infected with *Volutella* and *Humicola* and this was correlated with the lack of any extensive lesion development.

Further inoculation experiments were attempted using seedlings six weeks old growing in sterilized soil in conical flasks. The seeds were surface sterilized before being sown, and later the soil was inoculated with a culture solution containing fungal mycelium and spores, the soil of the controls being inoculated with sterile culture solution.

Growth of the seedlings in the flasks was poor, partly owing to the light conditions and possibly also to the effects of the autoclaved soil. Some differences were noticed between roots treated with different fungi, and a few reisolations were made. Soil isolations indicated that the fungal inoculants did not grow extensively in the sterilized soil, *Chaetomium*, *Colletotrichum* and *Humicola* appeared to be most active.

Small brown lesions at the base of the stem appeared on seedlings inoculated with *Petriella*, *Colletotrichum* and K. Browning of some of the root tips occurred on those inoculated with *Chaetomium*, while in soil inoculated with K the young lateral roots were becoming brown and rotted.

The fungi were reisolated from about one-quarter of the cultures made from roots treated with *Petriella*, *Chaetomium* and K, *Colletotrichum* and *Humicola* were reisolated from a few seedlings.

The results of inoculation experiments on seedling tomato plants, suggests that more than one fungus may exert an adverse effect on the roots. *Colletotrichum atramentarium*, which is known to cause disease on older roots, does not seem to be so effective against seedling roots as the three fungi *Chaetomium cochliodes*, *Petriella asymmetrica* and K. Both *C. cochliodes* and *P. asymmetrica* were found frequently associated with tomato roots during the first three months after planting out, while K was isolated from diseased roots throughout the season. It seems probable that these fungi when present in the soil may be partly responsible for the rotting of the roots, acting either pathogenically or as secondary saprophytic organisms, and further experiments to test the pathogenicity of the fungi isolated from tomato roots are to be carried out.

The fungus K resembles in its chief morphological characteristics a pathogen isolated by Berkeley and Richardson¹ from corky roots and basal rot tissue of tomatoes in Canada, but other fungi associated with root lesions in this case differ from those found at Cheshunt. The difference in the fungal flora of the roots combined with the similarity of the lesions described, suggests that brown root rot may not be due to any one specific agent. The fact that the disease is far more severe in unsterilized as opposed to sterilized soil, however, does suggest that micro-organisms play a primary part in its development.

Thanks are due to Mr. S. J. Hughes, of the Commonwealth Mycological Institute, for identifying some of the fungi.

REFERENCE

- ¹ Berkeley and Richardson, *Phytopath.* 1944, **34**, 615.



FIG. 1

a) The mosaic symptoms were produced on the moistened leaves by tobacco dust on the fingers.

(b) Control plant.

4. VIRUS DISEASES

By R. HOWLES

(1) Tobacco as a Source of Virus Infection in the Tomato Crop

Tobacco has long been regarded as a source from which the tomato crop is infected with virus disease.

Ainsworth¹ showed here in 1934 that tobacco mosaic virus occurs in pipe and cigarette tobacco and growers were advised to forbid smoking in tomato houses.

During the course of his investigations regarding the importance of virus-free tomato seeds to the glasshouse industry, the Director has observed repeatedly that where non-smokers only were employed on a nursery tomato crops from virus-free seed remained free from virus diseases throughout the whole of the season.

There is to-day a tendency to doubt the importance of tobacco smoking as a cause of tomato mosaic and so it seemed that the transmission of virus diseases by this means should be investigated more fully.

Infection could arise from virus on the hands picked up from the wet end of a cigarette, by a pipe-smoker rolling tobacco in the palm of the hand or from the mouthpiece of a pipe. Tobacco, especially tobacco dust, could soil the hands, particularly in the case of those who finger tobacco, namely, persons who make their cigarettes or smoke pipes, and the pruning knife could be contaminated with dust by keeping it and tobacco in the same pocket. The wet outside portion of the hose used for watering could collect tobacco dust from the hands. Tobacco deposited on the soil might be harmful.

As a start the debris from the bottoms of pockets was tested for virus in the case of tomato workers from several nurseries. Samples from 15 pockets contained virus, those from four did not.

Out of 41 nursery workers 22 smoked factory-made and 15 home-made cigarettes and there were four pipe-smokers who each rolled tobacco in the hands. Twenty-five nursery hands out of 59 kept the penknife and tobacco in the same pocket.

The results of experiments carried out here this year show that the tobacco-smoking habit makes virus available for causing infection in the tomato. Our experiments attempted to discover if infection could arise from:—

- (a) The wet end of a cigarette;
- (b) Rolling tobacco in the palm of the hand;
- (c) Fingering tobacco;
- (d) A knife which had been in contact with tobacco;
- (e) Water which had been in contact with tobacco, no grinding being involved.

A brand of tobacco which contained a considerable amount of virus was used. The *Nicotiana glutinosa* test plants were at the 5-10 foliage leaf stage.

(a) Infection from the wet end of a cigarette

Virus capable of acting as a source of infection was sought for at the wet end of a cigarette. The cigarette end was rubbed over the wetted leaves of a *N. glutinosa* plant, without the use of carborundum which might have ground up the tobacco. The tobacco in the butt-end was then ground in water and the suspension applied to another *N. glutinosa* plant. The experiment was performed 15 times. In each case there was virus in the tobacco. Twenty lesions were counted on the plants treated with the cigarette ends.

(b) Infection from rolling tobacco in the palm of the hand

Coarse tobacco was rubbed in the palm of the hand by means of the fingers. The palm was stroked with moistened cotton wool and a *N. glutinosa* plant was treated with the surface which touched the palm. The experiment was repeated nine times. There was no evidence of infection. Using tobacco dust kindly supplied by the manufacturers of the brand of tobacco the experiment was repeated 20 times. Seven lesions developed.

(c) Infection from fingering tobacco

After fingering coarse tobacco for three minutes the moistened leaves of a *N. glutinosa* plant were treated with the area which had touched the tobacco. The experiment was repeated nine times. No lesions developed. Using tobacco dust and young *Nicotiana tabacum* plants the experiment was carried out 20 times. The plants were treated on November 29th, 1949. Forty-one days later five plants out of 20 were infected. Fig. I illustrates the experiment.

(d) **Infection from a knife which had been in contact with tobacco**

A virus-free knife was put in contact with coarse tobacco. The knife was afterwards rubbed with wet cotton wool and then the leaves of a *N. glutinosa* plant were stroked with the part of the cotton wool which had touched the knife. This experiment was performed 10 times. No lesion developed. The experiment was repeated 30 times using tobacco dust. Tobacco dust was present on the blade after removal from the tobacco. Six lesions arose.

(e) **Infection from water which had been in contact with tobacco, no grinding being involved**

Ainsworth soaked tobacco in water, applied this inoculum to tobacco plants and obtained positive results. From his published work it is not clear if the virus exuded unaided from the tobacco: trituration, a process which could be avoided to a considerable extent on a nursery, may have expelled virus during treatment of the plant.

To decide this point coarse tobacco was gently mixed with distilled water. A day later the mixture was filtered through cotton wool. The filtrate was applied to nine tomato plants dusted with carborundum and at the six-foliage-leaf stage. Part of the mixture of tobacco and water was ground and then applied to a *N. glutinosa* plant to test for virus in the tobacco. The presence of virus in the filtrate is shown by the following results. The plants were treated on March 13th, 1949. Forty-seven days later six plants out of nine were infected. In another experiment the plants were treated on June 25th, 1949. Twenty days later four plants out of nine and no controls out of nine were infected. A confirmatory experiment was carried out using tobacco dust. The filtrate was applied to 15 *N. glutinosa* plants. Numerous lesions formed.

Field Trial

It was shown that no infection in tomato plants was obtained when just filter-tipped cigarettes were smoked.

Tomato seedlings were potted on July 15th, 1949. Fifty seedlings were potted by a person smoking filter-tipped cigarettes, another fifty by a non-smoker. Transplanting on August 18th and trimming on September 5th were done under same application of the differential conditions. The plants (50) potted, etc., by the smoker were tested for virus about October 18th. They were uninfected.

Conclusions

The non-filter-tipped cigarette smoker, particularly the person who makes his own cigarettes, the pipe-smoker and the worker who keeps the pruning knife in the tobacco pocket can introduce mosaic infection to the tomato crop.

These experiments do not go beyond what might be called laboratory studies, they do not prove there is sufficient virus under nursery conditions to cause extensive permanent infection: that could only be determined by field trials.

Further field trials with cigarettes fitted with filter tips are necessary, but the result of this single experiment suggests that this type of cigarette may reduce the danger of infection to some extent. It should be observed, however, that tomato growers who make their own cigarettes, even if they add filter tips, will still carry the infection on their hands, unless they wash them very thoroughly afterwards.

(2) Viricides

Last year it was shown here that 1 per cent. tannic acid in water acts as a spray viricide and can destroy mosaic virus before it enters the plant.² This work was continued, small field trials being carried out.

A viricide could also be used on the hands and pruning knife. They could be dipped periodically in some disinfectant—0.5 per cent. tannic acid solution or 2 per cent. solution of lysol, rinsing immediately afterwards in water to avoid harming the hands. At the suggestion of the Director it was decided also to try as a viricide for the hands a paste containing an anti-virus substance.

(a) Viricidal Sprays—Experiment 1

Thirty-six similar tomato plants each potted in a size 60 pot were used. The plants throughout the experiment were watered uniformly.

The terminal leaflets of the fourth foliage leaf from the base of the stem of each of six of the plants when at the six foliage leaf stage were inoculated with tomato aucuba mosaic virus. After dusting carborundum powder uniformly over the leaflets each one was stroked twice with cotton

wool soaked in a solution of the virus. The solution was prepared by grinding 1 gm. of infected *Nicotiana tabacum* in distilled water, filtering through cotton wool, rinsing the virus left behind into the cylinder, making the filtrate up to 200 ml. using distilled water throughout and finally mixing the solution.

Following the method of Roberts³ the 30 uninoculated potted plants were arranged in circles of five plants each, the pots almost touching. Three inoculated plants and three circles of plants were sprayed with a fine spreading spray of 1 per cent. tannic acid. The solution was not kept in the sprayer longer than necessary as the tannic acid attacks the iron of the casing. A sprayed inoculated plant was placed in the centre of each sprayed group and a non-sprayed potted inoculated plant in the centre of each non-sprayed group. Spraying was carried out once a week, the centre plant being removed and replaced. Since moulds grow readily in the solution fresh liquid was used each time. There was generally no spray injury other than a slight yellowing of a few leaves.

As the plants developed they were transplanted into larger pots.

One centre plant did not become infected. This fault was corrected in subsequent work. The rate of spread of symptoms of the disease to the outer plants was recorded.

Experiment 2

This experiment was a repetition of the last, using 50 plants. Twenty of the plants were inoculated and the first six uniformly mottled ones became the centre plants.

Experiment 3

This experiment was a third repetition.

The experiment was performed late in the year and so as only light and dark green mottling was likely to appear, symptoms which are often vague, it was decided to test periodically each outer plant and afterwards each outer uninfected one for virus. The tests were made as follows. A leaflet in a particular position relative to the base of the stem was cut off each plant. Tannic acid was removed from each sprayed leaflet by completely immersing it in about 75 ml. of distilled water in a 100 ml. beaker for one day. Each leaflet was ground in 10 ml. of distilled water. The suspension was applied, using cotton wool of a definite size, to all the leaves of a *Nicotiana glutinosa* plant. All the plants were similar at the five to seven foliage leaf stage and watered uniformly.

Using obviously sprayed leaflets from a centre plant and for comparison leaflets from a centre control it was shown that soaking as above freed leaflets from tannic acid.

A plant was regarded as infected when there were five or more than five lesions on its test plant. The results are tabulated in Table I.

TABLE I
EFFECT OF SPRAYING WITH 1 PER CENT. TANNIC ACID ON THE RATE OF SPREAD OF AUCUBA MOSAIC

Experi- ment	Date	Treatment	Number of infected* peripheral plants†	
			Sprayed Groups	Control Groups
1	2 May, 1949	Plants were inoculated.		
	6 May, 1949	Inoculated plants were placed in contact with uninoculated ones.		
2	8 June, 1949		2 out of 15	5 out of 10
	23 June, 1949		2 out of 15	5 out of 10
	27 June, 1949	Plants were inoculated.		
	7 July, 1949	Inoculated plants were placed in contact with uninoculated ones.		
	27 July, 1949		2 out of 15	5 out of 15
3	3 Aug., 1949		3 out of 15	6 out of 15
	10 Aug., 1949		6 out of 15	8 out of 15
	17 Aug., 1949		9 out of 15	9 out of 15
	30 Aug., 1949	Plants were inoculated.		
	17 Sept., 1949	Inoculated plants were placed in contact with uninoculated ones.		
	27 Sept., 1949		0 out of 15	0 out of 15
	7 Oct., 1949		0 out of 15	3 out of 15
	17 Oct., 1949		1 out of 15	5 out of 15
	28 Oct., 1949		9 out of 15	13 out of 15

* See the text.

† Plants previously found infected and not retested are included.

Conclusions

Spraying with 1 per cent. tannic acid once a week considerably delayed the spread of aucuba mosaic between tomato plants by contact. When the disease had passed to a third of the controls the spread in the case of the sprayed plants was 30 per cent. of this.

This occurred one month after first contact with the centre plants. In six months in a glasshouse one plant could be the source of infection of 110 ($\pi 6^2$) plants. This spread is rapid, but it is not rapid enough for spraying to be valueless.

The medical aspect of spraying is a question for consideration.

(b) Anti-virus paste for the hands and knife while trimming

An experiment was carried out to determine if covering the hands and trimming knife with paste containing an anti-virus substance would reduce the spread of virus disease.

A piece of india-rubber was halved and half of one of the halves covered with paste (*ung. aquosum*), containing an anti-virus substance non-injurious to the skin. 100 ml. of tomato aucuba mosaic virus solution were prepared and 50 ml. placed in each of two beakers. The pasted half of the piece of rubber was dipped completely in the liquid in one of the beakers and a similar half of the other piece of rubber dipped completely in the liquid in the other beaker. Each piece of rubber was shaken immediately after being wetted. Corresponding halves of the leaves of a *Nicotiana glutinosa* plant at the five to ten foliage leaf stage were now stroked with wetted cotton wool and then the treated end of one of the pieces of rubber. Similarly, the other halves were next wetted and then treated with the other piece of rubber. Immediately afterwards the pieces of rubber were washed with soap and water. Five plants were treated.

The experiment was repeated omitting the anti-virus substance.

The results are given in Table II.

TABLE II
THE EFFECTIVENESS OF PASTE ON THE HANDS AND KNIFE WHILE TRIMMING IN REDUCING THE SPREAD OF AUCUBA MOSAIC

Material Used	Experiment	Ml. of Virus Solution Prepared from a Gm. of Leaf	Number of Lesions	
			Following the Use of Paste	Control Value
Paste containing tannin {	1	200	1	4
	2	50	1	37
	3	50	5	17
Paste alone ... {	1	50	1	12
	2	33½	7	40

Conclusion and discussion

From the data in the table it follows that the paste alone would be a suitable substance for treating the hands and knife during trimming.

No anti-virus substance could be extracted out of the paste by means of water. It is possible that:—

1. The paste smothers the virus particles and prevents entry into the plant.
2. The paste blocks the fractured leaf hairs.
3. Virus adheres to the base.
4. The base destroys virus.

(3) Investigations in the therapy of virus-infected glasshouse plants

The chief published work up to 1948 on the inactivation of virus in vivo is that of Kunkel, Howles and Stoddard. Kunkel found that yellows in the peach, in periwinkle plants and in *Nicotiana rustica* plants could be cured by heat treatment. Howles showed that heat treatment of *Solanum capsicastrum* plants inoculated weakly with mild tobacco mosaic virus caused fewer plants to develop symptoms than in the controls. Destruction of the virus of X-disease in peach buds by immersing them in water solutions of chemicals was reported by Stoddard.

Methods of treatment which suggest themselves are:—

1. Seeds.
 - (a) X-ray treatment;
 - (b) Heat treatment;
 - (c) Chemical treatment;
 - (d) Subjection to conditions which cause fermentation.
2. Vegetative propagation.
 - (a) X-ray treatment.
 - (b) Heat treatment.
 - (c) Chemical treatment.
3. Growing plants.
 - (a) Heat treatment.;
 - (b) Chemical treatment.

Supersonic sound may have no effect because its destruction of virus is probably dependent on turbulence of the medium, although it should be tried on seed.

Attempts to inactivate the virus in virus-infected seed

Seed transmission of virus diseases occurs in the case of tomato mosaic, cucumber mosaic, lettuce mosaic and tobacco ringspot virus in the *Petunia*. Spotted wilt virus is not regarded as being seed transmitted in the tomato.

As a control measure it follows that one should use virus free seed. At the present this means seed from virus-free plants. Virus-free tomato seed is not much more expensive than ordinary tomato seed, so it may not be worth attempting to obtain clean seed by suitable treatment. It would, however, save labour, if some simpler method of producing virus-free seed of new varieties could be devised, instead of devoting so much care and attention to growing a virus-free crop.

X-ray treatment of tomato seeds

Lea⁴ reported that the dose of X-rays, wavelength 1.5Å, required to reduce the activity of tomato bushy stunt virus to 37 per cent. was 620,000 röntgens. In the case of tobacco mosaic virus Lea et Alia⁵ found that the dose of μ -rays of radium which reduced the lesion count to 37 per cent. was 250,000 röntgens. 1 mg. kept at 0° C. was irradiated with 7,700 röntgens of rays per hour.

In our experiments tomato seeds from plants infected with virus, chiefly mild tobacco mosaic virus (1947), were completely separated into single seeds and mixed for 15 minutes. Two 1,500 seed lots were weighed out: 100 seeds weigh 0.3 gm. One batch was kindly irradiated by Mr. F. R. George at the Hertford County Hospital. The seed received 400,000 röntgens at 60 kilovolts.

The appearance of the seed after irradiation was normal. Two hundred seeds from each batch were sown. The number of seeds which ultimately germinated was: Treated seed, 179; control seed, 172. Seedlings from treated seeds were normal. For corresponding seedlings the average weight of a seedling was 0.086 gm. in the case of treated seed, and 0.079 gm. for control seed. Irradiation of seed increased slightly the percentage germination and seedling weight. The increase was greater in the case of the earlier sowings.

The amount of virus in the irradiated seed and the untreated seed was compared by the half-leaf method. In each case 200 seeds were ground finely with 10 ml. of water. The liquids were applied to 10 (normal) *Nicotiana glutinosa* plants at about the 10 foliage leaf stage as follows. Carborundum was scattered uniformly on each leaf, and then each liquid was applied with a small piece of cotton wool, the pieces being equal in size. Looking (downwards on the plant and) outwards from the main stem the left hand of each leaf on the main stem was rubbed with liquid containing ground irradiated seed and the other half of the leaf with the other liquid. The leaf was rubbed from the petiole to the leaf tip. Liquid was applied to each half of the leaves of one plant completely before being applied to the next and all corresponding halves were treated before the others, in each case working clockwise round the stem. The plants were rinsed with water one hour later. They were watered uniformly (100 ml. of water per plant every three days) beginning one day before treatment.

Results

Three experiments were carried out. The data obtained are contained in Table III.

TABLE III
EFFECT OF X-RAY TREATMENT OF VIRUS INFECTED TOMATO SEED ON THE VIRUS CONTENT

Date of Treatment of Test Plants	Date of Counting Lesions	Number of Lesions per 10 Plants	
		Irradiated Seed	Control Seed
5 Jan., 1949	8 Jan., 1949	412	344
	9 Jan., 1949	562	491
12 Jan., 1949	15 Jan., 1949	247	237
	16 Jan., 1949	487	475
19 Jan., 1949	23 Jan., 1949	102	106
	24 Jan., 1949	149	146
	Total: $\div 2$	979	899

Conclusion

Treatment of virus-infected tomato seed with 400,000 röntgens of X-rays had no inactivating effect on the virus content.

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5. INVESTIGATIONS ON MICROBIAL ANTAGONISM AND ANTIBIOTIC SUBSTANCES. PROGRESS REPORT.

E. GROSSBARD.

The production of antibiotic substances in the soil.

I (A) *Penicillium patulum* in autoclaved soil

Experiments on the production of an antibiotic substance in autoclaved soil, i.e. in soil which was completely sterile, were repeated in order to obtain further confirmation of previous results. This work has been completed and a paper prepared for publication. Experimental details were given in previous reports.¹ In 1949 modifications of the assay technique were introduced. It was found that on the Difco beef agar medium used previously the growth of one of the bacterial test organisms namely *Bacterium carotovorum* (*B. phytophthorum*) was poor: the zones of inhibition ill defined and hard to read. After testing several media the best results were obtained when adding 0.3 per cent. Difco yeast extract to the standard Difco beef agar. Although the diameter of the area of inhibition tended to be smaller on this medium, especially in the case of the second test organism *Bacillus mycoides*, the above medium was chosen for further experiments because the zones of inhibition were much clearer with both test organisms and thus reading was greatly facilitated.

The antibacterial activity of the soil centrifugates was expressed in terms of the "patulin equivalent" which is that concentration of a patulin solution which gives the same diameter of the area of inhibition as that formed by a given soil centrifugate under test. Again the cylinder plate method was used. The choice of patulin as a standard has some justification. Birkinshaw *et al.*² showed that *P. patulum* produced on synthetic media such as Raulin-Thom solution an antibiotic substance termed patulin (clavatin, clavacin, claviformin, expansine) and which was identified by these workers as anhydro-3-hydroxymethylene-tetrahydro-1 : 4 pyrone-2-carboxylic acid. Although no chemical analysis was made of the inhibitive agent present in the soil centrifugates, the similarity in their biological activity with that of patulin suggests that this antibiotic may be present in the soil centrifugates. The standard was prepared by dissolving purified patulin in centrifugates made from the same soil as was used for the inoculation experiments with *P. patulum*.

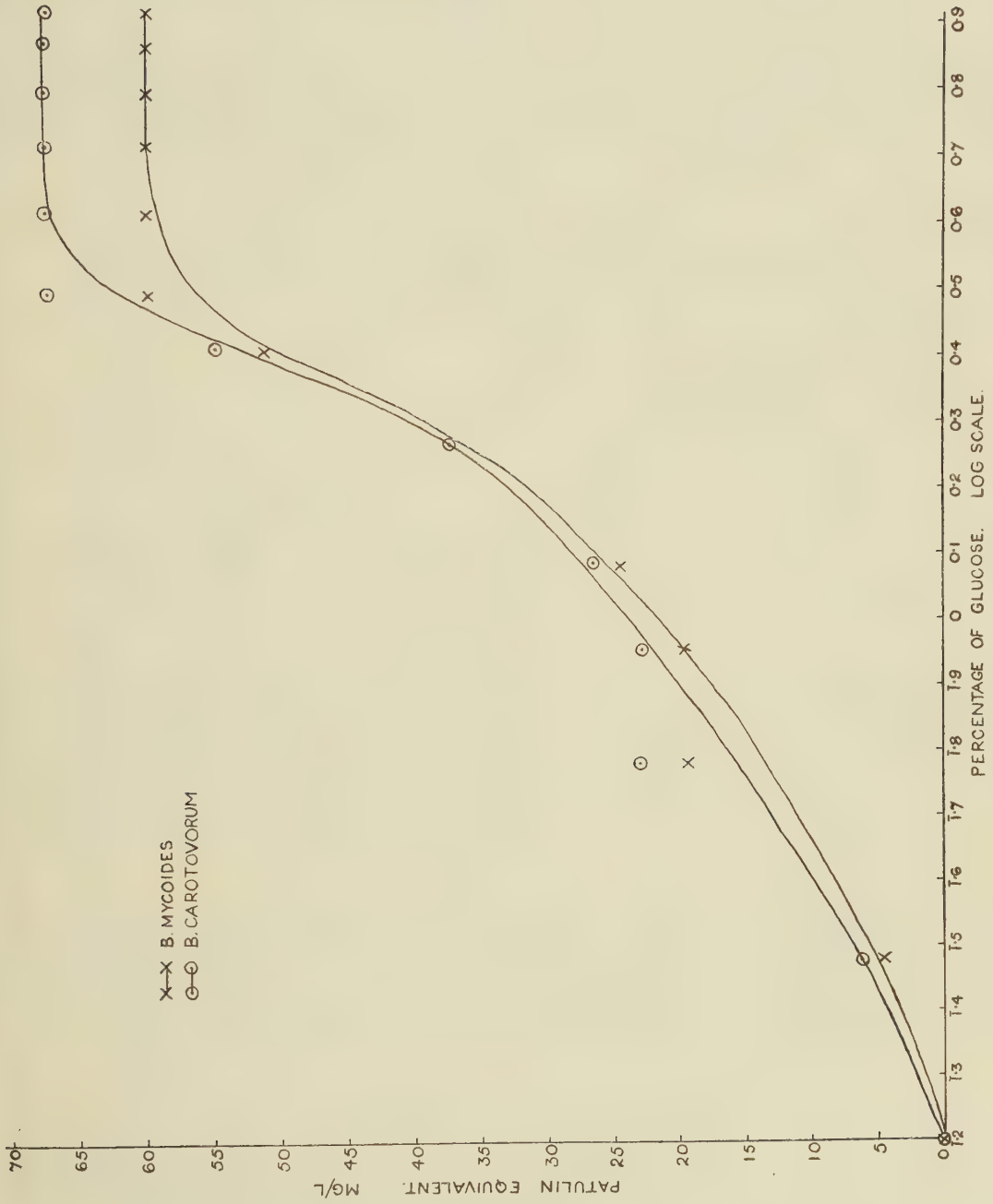


FIG. 1A

A comparison of the sensitivity of *B. mycoides* versus *B. carotovorum* to centrifugates from soils containing glucose and inoculated with *P. patulum*.

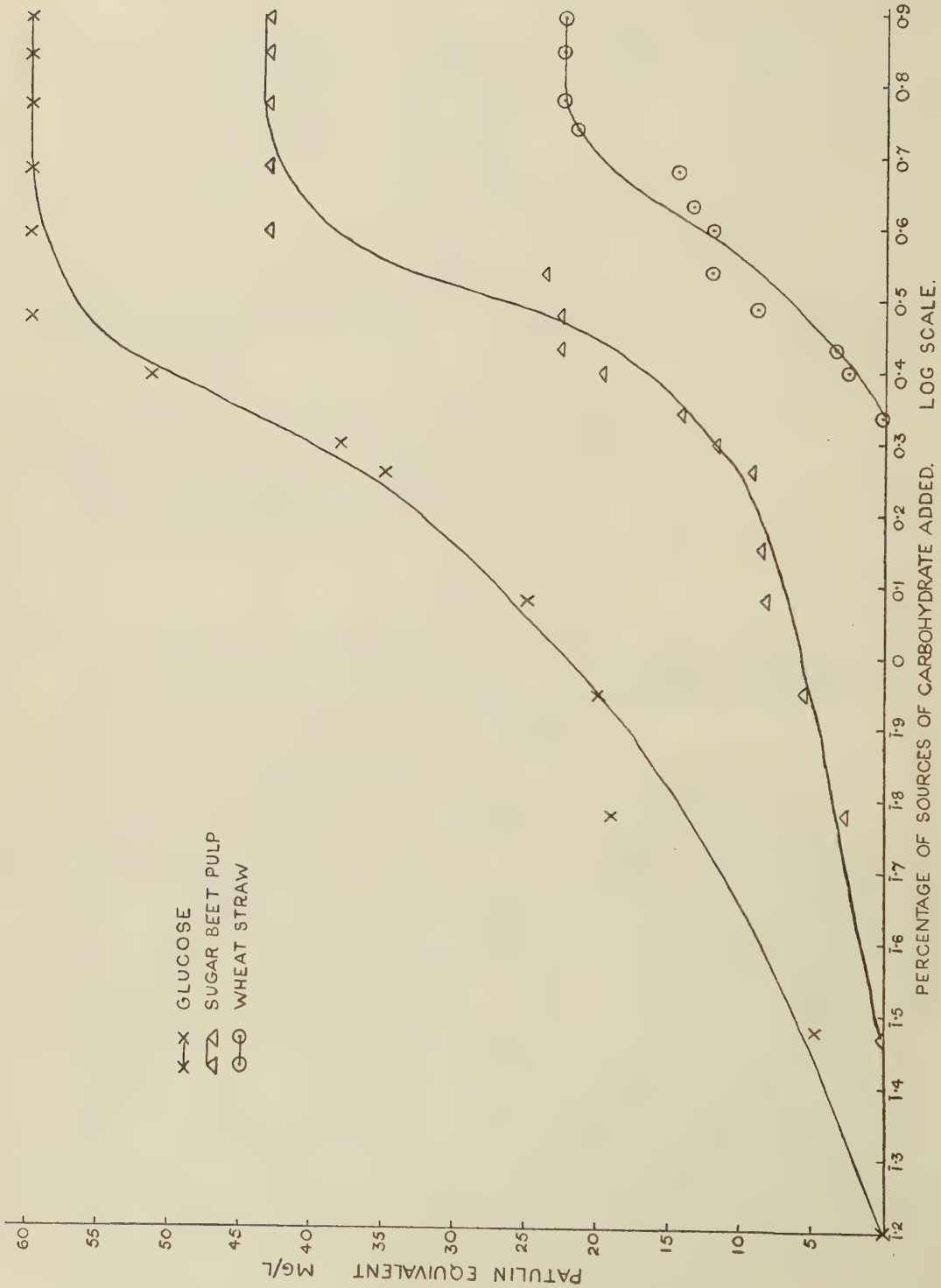


FIG. 1B

The relation between percentage of sources of carbohydrate added to autoclaved soil, inoculated with *P. patulum*, and the antibacterial activity of the centrifugates of these soils.

The results as represented in Fig. 1 confirm those reported before, namely that an antibiotic substance can be produced by *P. patulum* in autoclaved soil with which a source of carbohydrate is incorporated. An increase in the proportion of the organic material led at first to an increase in biological activity of the centrifugates. However, the maximum was reached fairly soon and after this any further increase in the supply of carbohydrate did not seem to have any effect on antibiotic activity. In the case of glucose the minimum and maximum points were comparatively close to each other.

For testing antifungal activity of the soil centrifugates, in addition to *Phytophthora parasitica* a second test organism, namely *Thielaviopsis basicola* was used. This is a soil-borne pathogen which grows and spores easily in pure cultures and is very sensitive to patulin. Antifungal assays were confined to centrifugates made from soils to which the three sources of carbohydrate were added at two levels only, namely 2.5 per cent. and 4 per cent. of the total weight.

TABLE I

THE ANTIFUNGAL ACTIVITY OF CENTRIFUGATES FROM AUTOCLAVED SOILS CONTAINING EITHER GLUCOSE OR SUGAR BEET PULP OR WHEAT STRAW AND INOCULATED WITH *P. patulum*

Centrifugates from	Concentration required for Complete Inhibition	
	Test Organisms	
	<i>Phytophthora parasitica</i>	<i>Thielaviopsis Basicola</i>
Soil only	Growth	Stimulation
Soil + Glucose 2.5%	1 : 100	1 : 100
" + " 4%	1 : 100	1 : 100
Soil + Sugar Beet Pulp 2.5%	1 : 80	1 : 40
" + " 4%	1 : 100	1 : 80
Soil + Straw 2.5%	1 : 10	1 : 5
" + " 4%	1 : 20	1 : 10
Control: patulin dissolved in sterile soil soil centrifugates	1 : 160,000	1 : 80,000

Timothy grass as a fourth source of carbohydrate

Experiments were made using timothy grass as a fourth source of carbohydrate. Preliminary tests in 1948 with pure cultures of *P. patulum* on this grass had shown a somewhat greater activity of the metabolic fluid as compared with that of wheat straw cultures. Yet when timothy grass was incorporated with the soil results tended to be very variable and figures were often far below those from soil straw media. Owing to a shortage in the supply of this grass it was not possible to make sufficient replicates in order to assess finally the relative merits of timothy grass as a source of carbohydrate for antibiotic production in the soil.

(B) *P. patulum* in steamed soil

Preliminary results in 1948 showed that an antibiotic substance was produced by *P. patulum* in soil which had been steamed once for 30 minutes at 100° C. Repeating these experiments, glucose, molassed sugar beet pulp and wheat straw were used respectively as sources of carbohydrate at two levels, namely 2.5 per cent. and 4 per cent. of the total weight.

The figures in Table II show that while the addition of glucose or sugar beet pulp to the soil induced antibiotic production, the incorporation of wheat straw yielded negative results.

(C) *P. patulum* in non-sterile soil

Investigations so far were confined to either completely or partially sterilized soil. In 1949 small-scale experiments were made using ordinary soil. The technique applied was similar to that used in trials with steamed soil. Three test organisms were used, namely *B. mycoides*, *B. carotovorum* (*B. phytophthorum*) and *B. coli*. The results of the assays were negative. It was not possible to detect an inhibitive agent in the soil centrifugates with the exception of 2 per cent. of the assays when *B. coli* was used as test organism. In this case a very small halo of weak partial inhibition was noted, but as these observations were also made with the blanks no significance could be attached to these results except that they indicated that the soil contained an agent slightly inhibitive to *B. coli*. This observation had been made before in a few isolated instances.

TABLE II
A COMPARISON OF THE ANTIBACTERIAL ACTIVITY OF CENTRIFUGATES FROM PARTIALLY VERSUS COMPLETELY STERILISED SOILS
The soils contained one of three different sources of carbohydrate and were inoculated with *P. patulum*

Centrifugates from	Test Organisms				
	<i>B. mycoides</i>		<i>B. carotovorum</i>		
	Soil		Reduction in Yield	Soil	
	Steamed	Autoclaved		Steamed	Autoclaved
Soil + Glucose 2.5% ...	*31.62	51.5	38.7	44.67	55.0
" + " 4% ...	31.62	60.0	47.3	44.67	73.8
" + Sugar Beet Pulp 2.5% ...	10.69	22.9	53.4	11.69	22.39
" + " 4% ...	16.18	43.75	63.1	18.58	50.23
" + Wheat Straw 2.5% ...	No activity but growth stimulation			Mostly no activity. Occasionally weak partial inhibition†	
" + " 4% ...					

† Areas of weak partial inhibition especially of surface colonies of about 11-12 mm. diameter are occasionally produced by centrifugates from untreated soils also.

* Patulin equivalent Mg/l.

TABLE III
A COMPARISON OF THE ANTIBACTERIAL ACTIVITY OF CENTRIFUGATES FROM AUTOCLAVED SOILS CONTAINING THREE SOURCES OF CARBOHYDRATES
AND INOCULATED WITH EITHER *Aspergillus Terreus* OR *Penicillium patulum*

Centrifugates from	Inoculated with <i>A. terreus</i>				Inoculated with <i>P. patulum</i>			
	<i>B. mycoides</i>		<i>B. carotovorum</i>		<i>B. mycoides</i>		<i>B. carotovorum</i>	
	Inhibition	Diameter of Area of Inhibition	Inhibition	Diameter of Area of Inhibition	Inhibition	Diameter of Area of Inhibition	Inhibition	Diameter of Area of Inhibition
Soil only ...	Growth stimulation	—	*None or weak partial	—	Growth stimulation	—	None or weak partial	—
Soil + Glucose 2.5% ...	Complete	13.1	Partial to almost complete	12	Complete	28.2	Complete	29.0
" + " 4% ...	"	17.2	Complete	16	"	30.0	"	31.5
Soil + Sugar Beet Pulp 2.5% ...	Complete	13.3	*Weak partial	—	Complete	18	Complete	19.5
" + " 4% ...	"	15.7	"	—	"	29	"	29.6
Soil + Straw 2.5% ...	Growth stimulation	—	None	—	Complete	12.7	Complete	13.1
" + " 4% ...	Complete	12	"	—	"	17.8	"	18.2

* Areas of weak partial inhibition especially of surface colonies of about 11-12 mm. diameter are occasionally produced by centrifugates from any soil.

II. *Aspergillus terreus* in autoclaved soil

Aspergillus terreus is a soil organism of which several strains had been isolated. The species produces several antibiotics, namely citrinin, geodin, itaconic acid, patulin and terreic acid. The antagonistic action on *Botrytis allii* of two strains (N.C.T.C. No. 981 and 3911) had been reported by Brian and Hemming.³ Preliminary experiments with this fungus, using strain N.C.T.C. No. 3911 have shown that like *P. patulum* this fungus has the property of producing an antibiotic substance in autoclaved soil. Experiments on a larger scale were carried out in 1949 using the same technique as employed in experiments with *P. patulum*. Again three sources of carbohydrate were incorporated, namely glucose, molassed sugar beet pulp and wheat straw at 2.5 per cent. and 4 per cent. levels. The results were compared with those obtained from centrifugates from similar soils but inoculated with *P. patulum* and are set out in Table III.

The above figures show the antibacterial activity of the antibiotic formed by *A. terreus*. It should be noted that activity is considerably lower than that from *P. patulum* centrifugates especially when *A. terreus* was cultured on a soil and straw medium. The latter result is somewhat unexpected in view of the fact that Norman⁴ reported the isolation of *A. terreus* from decomposing straw, demonstrating that this fungus takes an active part in the break-down of the straw. Although Norman used oat straw while our experiments were made with wheat straw, one might nevertheless have expected that efficiency in the decomposition of straw might lead to a good yield of an antibiotic substance.

Preliminary tests were made for antifungal activity using *P. parasitica* and *T. basicola* as test organisms. It appears that the growth of these fungi is inhibited only to a slight degree. After a lag period some growth takes place. These results require further confirmation.

A chemical analysis of the antibiotic present in the above soil centrifugates has not been carried out. Because of the difference in the sensitivity of the test organisms it is unlikely that a substance similar to that produced by *P. patulum* cultures on similar substrata had been formed. While *B. mycoides* was sensitive to all centrifugates *B. carotovorum* showed resistance with one exception. Centrifugates from soils plus glucose induced zones of inhibition on *B. carotovorum* plates. As had been pointed out before *A. terreus* produces patulin. It is suggestive that only in the presence of glucose is patulin produced by this particular strain of *A. terreus* in the soil. If it is manufactured at all then its concentration is considerably lower than that formed by *P. patulum*.

III. Other fungi producing antibiotic substances

Six species of *Penicillia* were isolated from the soil. None were identified. In a preliminary test these organisms were inoculated into sterile centrifugates made from autoclaved soils to which glucose, molassed sugar beet pulp or wheat straw was added respectively. Only two species called A and B gave positive results. Further confirmatory experiments are in progress.

Discussion

The purpose of the fundamental investigations into the question of the possible production of antibiotics in the soil is an important one. They may lead to a practical method of inducing in the soil the formation of a specific antibiotic active against one or several soil-borne plant pathogens. If this were possible the disease-producing agent could be controlled at its source. The investigations should also help to throw some light on the mechanism of microbial antagonism, of which little is known.

There has been some controversy⁵ as to whether an antibiotic substance could be manufactured on so poor a substratum as the soil because although most antibiotic producing micro-organisms live in the soil, which is thus their natural substratum, it does not follow that an antibiotic will be manufactured under these conditions. It had been clearly established that certain media may support luxuriant growth of an organism without the formation of the antibiotic usually associated with it. The nutritional requirements for the production of an antibiotic substance in the laboratory or in the factory are very complex and highly specific. It is for these well justified reasons that many workers and in particular Waksman⁶ stated that micro-organisms would hardly have an opportunity to produce an antibiotic in the type of poor medium provided by soil.

The investigations carried out here were designed to obtain more information on this problem. On the evidence obtained it can be stated that at least in the case of the fungi *P. patulum* and *A. terreus*, and, as preliminary trials suggested, in the case of the two unidentified soil penicillia, the soil as such is not inhibitive to the formation of an antibiotic substance. It is claimed that, provided an adequate supply of carbohydrate can be added to the soil, this substratum provides all other nutrients required for antibiotic production under the conditions of our experiment. The missing

carbohydrate could be most effectively supplied by glucose of which only a small quantity will be required. It gave the most satisfactory results in the case of all four fungi. The results suggest that carbohydrate decomposition is one of the essential factors in antibiotic production in the soil. Addition of carbohydrates to the soil might encourage antibiotic manufacture by many other soil micro-organisms as well but it appears that this requirement is not a general one. Preliminary experiments just begun here with *actinomyces* indicate that other nutrients may play a more important part than carbohydrates.

Glucose has no practical application as a manure, but the use of sugar beet pulp and wheat straw may be a practical proposition. In the reports for 1946, 1947 and 1948 several other plant materials that could be used as manures were listed.

It is important to note that an antibiotic substance was produced also in a partially sterilized soil in which spore-forming bacteria must have survived and multiplied rapidly, thus competing for the available nutrients. In spite of this the antibiotic was formed but only in the presence of glucose and molassed sugar beet pulp. This suggests that not every type of microbial competition necessarily interferes with antibiotic production and persistence, as is believed by several workers in this field. As the partial sterilization of the soil is a routine measure in the glasshouse industry the possibilities of a practical application of the above results are worth noting especially as a means of preventing re-infection of freshly-steamed glasshouse soils. In this connection an experiment reported in 1948 should be mentioned. It was attempted to arrest the spread of damping-off of tomato seedlings by the inoculation of soils steamed by the usual commercial methods, with *P. patulum* and the simultaneous incorporation with glucose, molassed sugar beet pulp and wheat straw. While all other treatments were either ineffective or even stimulated the disease the molassed sugar beet pulp treatment reduced the spread of the disease from a centre of infection to a considerable degree. Although the results of this trial contradicted those obtained in previous trials, it is interesting to note that even in an experiment which gave such negative results on the whole, the sugar beet pulp treatment was positive.

The incorporation of wheat straw in the case of partial sterilization also gave negative results in the in-vitro experiments. This can be explained in two ways: (1) During the process of autoclaving chemical changes take place which make the various carbohydrate components of the straw more easily available for micro-organisms. It is possible that when steaming for 30 minutes only at 100° C. this process of carbohydrate break-down has not gone far enough. (2) The micro-flora developing on the soil-straw medium might be different in its composition from that on either the soil-glucose or the soil-sugar beet pulp medium. Organisms might have developed which interfered with both the production and the persistence of an antibiotic.

The ultimate aim of this research, however, is to induce antibiotic formation in non-sterile soil and thus dispense with the expensive and cumbersome control measures which have to be carried out at present. It is hoped to replace these methods by inoculating non-sterile soils with one or a combination of antibiotic producing organisms coupled with a specific manurial programme designed to promote antibiotic formation and thus encourage a specific form of microbial antagonism. Although it has been shown here that it is possible to induce artificially the production of an antibiotic substance in completely and partially sterilized soil, great caution should be employed in drawing an analogy between results obtained in sterilized soil with those to be expected in non-sterile soil in the field.

Our efforts here to isolate an antibiotic substance from non-sterile soil inoculated with *P. patulum* have not met with success. However, in view of reports of other workers one might assume that one reason for our failure was the inadequacy of the extraction methods used. These consisted merely in the removal of the soil liquid by centrifuging. This technique is simple and quite adequate when the antibiotic is present in large concentrations but may fail when the inhibitory agent is present in great dilution only, as will be the case in non-sterile soil. Waksman and Woodruff⁷ have demonstrated the presence of a substance of the actinomycin type in ether extracts from certain soils, which inhibited the growth of certain bacteria in vitro. (Actinomycin is an antibiotic produced by *Streptomyces antibioticus*). Jacobs and Nandi using a modification of the alcohol displacement method extracted from non-sterile soil treated with glucose, straw and other carbon compounds an antibiotic substance at very low concentrations. The results of this latter work supports the suggestion made in the Annual Report for 1947 that the effect of glucose or any source of carbohydrate incorporated with the soil is not limited to a qualitative and quantitative modification of the microflora, but that in addition the formation of inhibiting agents of an antimicrobial nature may be encouraged.

Investigations into the possible injury of tomato seedlings by *P. patulum*

Gaumann *et al.*⁹ and Wright¹⁰ reported that patulin has a toxic effect on plants. Experiments carried out here showed that spraying the soil in boxes containing tomato seedlings in the first leaf



FIG. 2
The antagonistic action of *S. griseus* on *P. parasitica*.



FIG. 3
The antagonistic action of a species of *Penicillium* on *C. atramentarium*.

stage with a patulin solution of 1 : 1,000 caused injury to the seedlings. A constriction was observed on the stem approximately $\frac{1}{2}$ in. above soil level at which point the seedlings collapsed. The constriction was occasionally observed near the growing point. At lower concentrations, however, this effect was not noted. On the other hand, when the soil was sprayed with a solution of patulin at the phytotoxic concentration of 1 : 1,000 and seed sowing was carried out one week later the resulting seedlings were healthy and developed in the normal way.

In 1948 an experiment on the control of damping-off was described in which it was attempted to check the spread of the disease by the inoculation of soils with *P. patulum* and by manuring with the three sources of carbohydrate mentioned before. It was found in this trial that treatments, which in previous experiments brought about a certain measure of control of the disease, increased the incidence of damping-off in this case. In view of the above observations on the phytotoxicity of patulin it was feared that in 1948 an accumulation of the antibiotic presumed to be formed in the soil, took place and that its concentration was beyond the point tolerated by the seedlings. In order to test this hypothesis an experiment was designed in which soils were inoculated with *P. patulum* but not with *P. parasitica*. The technique employed was similar to that described for the damping-off experiment in the Annual Report for 1948. Results are given in Table IV.

TABLE IV
MEAN PERCENTAGE OF TOMATO SEEDLINGS EMERGED

Substratum	Inoculated with <i>P. patulum</i>	Not inoculated with <i>P. patulum</i>
(1) Soil only	97	95
(2) „ + Glucose (2.5%)	89	90
(3) „ + Sugar Beet Pulp (2.5%)	91	88
(4) „ + Straw (2.5%)	93	94
(5) „ + „ (5%)	86	84
(6) „ + „ (2.5%) + Glucose (2.5%)	88	87

Note the reduction in the emergence of tomato seedlings in soils containing carbohydrate sources irrespective of the presence of *P. patulum*.

The figures in Table IV indicate that there was no significant difference in the rate of emergence between the treatments and the blanks. The plants were removed from the boxes 10 weeks after emergence. Throughout this period of observation no injury was noted on the plants growing in soils inoculated with *P. patulum*. It may therefore be concluded that under the condition of this experiment the presence of *P. patulum* in the soil had no injurious effect on the tomato seedlings.

The antagonistic action of *Streptomyces griseus* on *Phytophthora parasitica*

Attempts to control damping-off of tomato seedlings by the antagonistic action of *P. patulum* gave contradictory results. For this reason it was felt advisable to search for other antagonists which might possibly give more consistent results. These investigations were started on two different lines.

- (1) Soil organisms known to produce antifungal substances were tested for their possible antagonism to *P. parasitica*.
- (2) An attempt was made to find such organisms by isolations made from the rhizosphere of healthy tomato seedlings growing in infected soil.

In a preliminary screening test of such organisms which were known to produce antibiotics *Streptomyces griseus* gave the most promising results. This effect is illustrated in Fig. 2. Both organisms were inoculated at opposite points in a potato glucose agar. Two types of blanks were set up. On one plate (c) only *P. parasitica* was introduced, on plate (b) a second fungus was inoculated at the opposite edge of the plate. This fungus, an unidentified species of *Penicillium*, was found not to antagonise *P. parasitica*. The antagonistic effect of *S. griseus* is shown in the formation of a straight line by the colony of *P. parasitica*. Growth had stopped at a considerable distance away from the *S. griseus* colony. The photograph was taken two months after inoculation. While on plate (c) the growth of *P. parasitica* filled the whole plate rapidly, on plate (b) both colonies grew steadily towards each other until they established contact, both filling the entire space of the plate thus illustrating absence of any antagonistic action. The formation of a straight line by the colonies of inhibited fungi had been observed with other organisms as well. Fig. 3 shows this effect with *Colletotrichum atramentarium* antagonised by an unidentified species of *Penicillium*. In other instances *P. parasitica* responded to the antagonistic action of *S. griseus* by forming a halo around the *S. griseus* colony, a zone free of fungal growth of varying diameter.

Experiments are in progress to investigate whether *S. griseus* would form an antibiotic substance in the soil. Both sterile and non-sterile soils were used in these experiments. As reported in 1947 *S. griseus* does not form an antibiotic substance when grown on wheat straw, thus the methods used to induce antibiotic formation in the soil by *P. patulum* will have to be modified. As results are not yet available experimental details will be given in a later publication.

Isolation of antagonistic organisms from the rhizosphere of healthy tomato seedlings

In the report for 1948 an experiment was described on the control of damping-off of tomato seedlings. Throughout the experiment a number of seedlings remained healthy in both the treatments and the blanks respectively.

Lochhead and Landerkin¹¹ had shown that the microflora of the rhizosphere differs from that of the surrounding soil. They hold that "the relative incidence of organisms with antibiotic properties towards a variety of organisms is different in the rhizosphere from that in control soils." In view of Lochhead's work an experiment was begun here to investigate whether there was any difference between the microflora in the rhizosphere of diseased seedlings from that of healthy seedlings within one and the same variety of tomatoes, in short whether disease resistance among individual plants of the same variety was in any way associated with a difference in the composition of the microbial population in the vicinity of the root system of sensitive and resistant plants respectively. In this attempted census only those organisms were taken into account which showed antagonism to *P. parasitica*. These isolations were made from the blanks taken from the damping-off experiments of 1948 in order to exclude the effect of any possible modification of the microflora due to the presence of *P. patulum*.

In view of the great labour involved in isolating and testing the numerous organisms this census is far from completion. From the rhizosphere of both healthy and diseased seedlings bacteria were isolated that antagonised *P. parasitica* in-vitro. However, one actinomycete and two species of *Penicillium* were isolated from the rhizosphere of healthy seedlings which up to the present moment have not yet been encountered in the rhizosphere of diseased plants. As the screening of the rhizosphere population of diseased seedlings has not yet been completed, final conclusions should not be drawn as it is quite possible that the three antagonists will still be found in the rhizosphere of the diseased plants.

Applying the techniques used with *P. patulum* the three isolates (one actinomycete, two species of *Penicillium*) were inoculated into autoclaved soil in an endeavour to test their potential power of producing the antifungal substance in the soil. The results differed for each organism. While no antibiotic substance could be detected in the centrifugates from soils inoculated with the actinomycete and with one of the unidentified species of *Penicillium* respectively, the second species of this fungus gave clear zones of inhibition against *B. mycoides*.

Although these trials were merely preliminary ones and carried out on a very small scale, they do indicate that the power to produce an antibiotic substance on a soil medium containing a carbohydrate source is not a property of every soil organism and that the specificity observed when dealing with other media holds true for the soil as well.

Summary

(1) The work on the production of an antibiotic substance by *P. patulum* on completely or partially sterilised soil has been completed. It was shown that, provided a source of carbohydrate was added to the soil, this medium furnished all other nutrients required for antibiotic production by this fungus. No antibiotic could be isolated from non-sterile soil inoculated with *P. patulum*.

(2) *A. terreus*, like *P. patulum*, produced an antibiotic in sterilized soil but was less efficient in this respect than *P. patulum*.

(3) Experiments are described concerning a possible injurious effect of *P. patulum* on tomato seedlings. No toxic action was observed when seedlings were cultivated in soils inoculated with this fungus.

(4) It was shown that *S. griseus* antagonises *P. parasitica*.

(5) Preliminary work has been outlined on the "rhizosphere effect" in an attempt to assess a correlation between disease resistance of individual tomato seedlings within one variety and the composition of the microflora of the rhizosphere of healthy and diseased seedlings.

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III. ANIMAL PESTS

1. TOMATO LEAF-MINER (*Liriomyza solani*, Hering)

By E. R. SPEYER and W. J. PARR*

1. Habits and life history

In order to observe their habits, the flies were kept under lamp-glasses inverted over tomato seedlings 2 to 3 ins. in height grown in pots, and covered with muslin.

A single female or one pair of flies were confined under each glass at a time, and diluted syrup soaked up on a piece of rubber sponge was provided as a source of food additional to that which the flies might obtain from the plant.

When a plant was exposed to sunlight the female fly would become so preoccupied in making its characteristic punctures in the cotyledons and more expanded secondary leaves, that it became possible to dispose of the lampglass and observe the process of feeding and oviposition under considerable magnification with a binocular microscope.

At convenient intervals of time varying from a few hours to two days, the flies were caught and liberated upon a fresh plant. From series of plants thus obtained, the number of eggs deposited by a single female over known periods of time was determined with a greater or less degree of accuracy, either by direct observation of the larvæ shortly after hatching or by counts made of the full-grown larvæ when they vacated their mines in order to pupate.

Recording thermometers placed in the glasshouses in which the plants were kept enabled the period of incubation and later the duration of larval life to be determined at known ranges of temperature. Temperatures were similarly recorded in the glasshouses or in the laboratory in which the puparia obtained from these and other larvæ were kept.

A. Habits of the Adult Flies

(1) Methods of feeding

Male flies subsisted entirely upon syrup provided for them, and appeared to feed principally during the earlier hours of the morning. Even when in close company with them, males were never observed to feed at the punctures made in the foliage by the females: no important structural difference in the mouth-parts of the two sexes could, however, be discerned. Female flies would occasionally leave the foliage to feed upon syrup at about mid-day when high temperatures prevailed, but otherwise obtained their nourishment entirely from the feeding-pits, which they constructed by means of their ovipositor during the morning and early afternoon, in the following manner:— Having selected a suitable spot, either upon the upper or lower surface of a leaf, the fly applied the truncated extremity of the abdomen to the surface and elevated the fore part of the body in order to exert greater pressure. The horny spatulate ovipositor was protruded so as to pierce the surface obliquely and force its way more or less horizontally and in a backward direction through the plant tissues. Piston-like thrusts to either side were then imparted to the ovipositor, which was employed as a chisel to rupture the plant cells and create, within its radius of action, a circular space immediately beneath the leaf surface. This accomplished, the ovipositor was used in the manner of a pestle to press down, with circular movements, and squeeze out the liquid from the underlying cells. The cavity thus became filled with the expressed liquid, and the ovipositor was withdrawn into the body. After the extremity of the abdomen had been lifted from the surface of the leaf, there was no noticeable exudation of fluid from the puncture at the entrance to the pit. The fly was occupied for about five seconds during the whole of this operation, after which the anal segment with its pair of cerci was extended and retracted several times, seemingly for the purpose of cleaning the under-surface of the ovipositor.

Having moved a little backwards and tilted its head downwards, the fly now forced the whole of the proboscis, up to and including the short maxillary palpi, through the puncture and proceeded to suck up the fluid in the pit. As the amount of liquid diminished, the epidermis of the leaf covering the pit became concave, and after the pit had been emptied in the course of about 10 seconds, the fly was able to withdraw the mouthparts through the puncture only with considerable effort. Any solid material derived from the crushed cells upon the floor of the pit, which adhered to the proboscis, was cleaned off with the tarsi of the forelegs before the insect began to construct another pit.

After having extracted the fluid from several pits, the fly remained motionless for some minutes, during which it regurgitated the liquid in the form of a greenish globule, which rapidly grew larger so as to envelope the proximal portion of the maxillary palpi, and was then again gradually absorbed.

Feeding pits were made upon both the upper and under surface of cotyledons and secondary leaves, but the flies fed to a greater extent upon the upper surface on which, apparently on account of their greater depth and the more rapid desiccation of the epidermal cells covering them, the pits were more conspicuous: those upon the under surface of a cotyledon, indeed, became practically invisible not long after the fly had emptied them, and could be located only from the punctures under comparatively high magnification.

The diameter of a newly-constructed feeding-pit was about 0.35 mm. and that of the puncture upon its periphery about 0.1 mm. Measurements taken some five days later showed the diameter of the pits and punctures upon the upper surface of a cotyledon to have increased respectively to 0.36 mm. and 0.12 mm., the expansion being caused, naturally, by the growth of the foliage.

In confinement, the fly often made large numbers of pits within a limited area of leaf-surface—one was observed to construct 39 feeding-pits upon the upper surface of a secondary leaflet about 10 mm. in length and 5 mm. in greatest width, though just previously it had made only 11 pits in an irregular row covering a distance of some 10 mm. upon the under-surface of a cotyledon. Seventy-one feeding-pits were counted upon the upper surface of a secondary leaf after a fly had been at work upon it for a period of about four hours.

(2) Mating

It is improbable that the insect is capable of parthenogenetic reproduction. A female fly which emerged from the puparium on March 26th and was kept isolated, lived for 18 days, during which it made feeding-pits in the foliage of the seedlings provided, but the insect fed to a lesser extent than paired females and deposited no eggs.

The following observations were recorded when single pairs of flies were kept upon seedlings under lamp-glasses:—

(a) At 11.0 a.m. on April 5th a newly-emerged female was confined with a male which had emerged from the puparium on the previous day. The female made 26 feeding-pits upon a secondary leaf before mating took place on its upper surface at 4.50 p.m. The flies remained *in coitu* for slightly longer than 10 minutes, and were not observed to mate again before the male was found dead on April 8th. The female deposited the first egg on the third day after mating and continued to oviposit over a period of 10 days.

(b) At mid-day on April 7th a newly-emerged male was confined with a female which had emerged from the puparium on the previous day. The female fed upon the syrup provided, and made few feeding-pits in the foliage of the seedling during the remainder of the day. At 9.15 on the following morning the flies mated upon the upper surface of a secondary leaf, and remained *in coitu* for 10½ minutes: during the course of the day the female made feeding-pits, deposited at least three eggs in the foliage of the seedling, and continued to oviposit over a period of 10 days.

(c) At 12.30 p.m. on April 11th the male, which had emerged from the puparium four days and had mated with the female referred to in (b) three days previously, was confined with a newly-emerged female. The female made 15 feeding-pits upon the upper surface of a secondary leaf, where at 2.10 p.m. the male paired and remained *in coitu* with it for 10 minutes. The female began to deposit eggs on the following morning, and continued to oviposit over a period of eight days.

The male, which survived in all for seven days, was dead on the third day after it had mated with the second female.

These records indicated that the flies paired during the daytime upon the lower leaves of the plant; the female mated but once during its life, while the male was at least capable of pairing with a second female.

The short period of some 10 minutes during which the sexes remained *in coitu*, together with the monogamous habit of the female, would account for the flies seldom being observed in the act of mating in glasshouses. Whereas the males appeared to be unable to pair for some 24 hours, the females were ready to do so within a few hours after emergence from the puparium. In the case of one female which was mated not long after it had emerged from the puparium, a period of two days elapsed before it began to lay eggs: in that of another which had mated the day after emergence, the insect began to oviposit within a few hours after pairing.

(3) Oviposition

A female fly, captured in a glasshouse and kept with a male in a glass tube on the previous day, was liberated in a lamp-glass covering a tomato seedling on February 2nd. During the morning the fly made a number of feeding-pits upon one of the cotyledons. At mid-day after having made five feeding-pits in the upper surface of a secondary leaf, it was seen to construct a pit of rather a different nature. After the ovipositor had been forced obliquely through the surface, and pushed horizontally with one or two piston-like thrusts through the leaf tissues, it was kept fully extended for a few seconds and then slowly withdrawn, leaving an egg at the distal end of the tunnel thus formed. The insect withdrew the ovipositor without having pressed down the plant-cells, though afterwards it imbibed the small amount of sap which had collected in the tunnel between the puncture and the egg. A number of feeding-pits were made before another egg was laid, and after two hours 39 circular feeding-pits and three tubular pits each containing an egg were counted.

The egg-pits were about 0.35 mm. and the white eggs 0.25 mm. in length, with a diameter of about 0.15 mm.

It was observed later that very occasionally an egg was deposited upon the floor of a feeding-pit after the plant-cells had been pressed down by means of the ovipositor.

Over the period of 15 days during which it remained alive, this female deposited 70 eggs in the foliage of the seven plants which were successively provided for it, the count having been made at the time when the larvæ hatched from them and began to mine in the foliage. Fifteen of these eggs and larvæ were preserved for microscopic examination, and 49 puparia were subsequently obtained from the series of plants.

The number of eggs laid by three mated females kept under similar conditions in April, computed from the number of larvæ and puparia subsequently obtained from the series of plants provided for each of them, was respectively 40, 104 and 62, with an average of seven and a maximum of about 15 per diem. Apart from a small number laid in the cotyledons, all the eggs were deposited in the three oldest leaves upon each plant.

(4) Span of life

The average length of life of seven males provided with syrup, whether kept isolated in glass tubes covered with muslin or mated with females and confined under lamp-glasses inverted over tomato seedlings, was five days (range three to nine days): that for four females kept upon seedlings under lamp-glasses was 12 days (range nine to 15 days), and an unmated female survived for 18 days.

(5) Proportion of the sexes

Of a total of 143 flies which emerged from puparia from the middle of March to the end of June, 71 were males and 72 were females. In collections made at random in glasshouses over much the same period in 1948, females were more than twice as numerous as males.

* See also Growers Notes p. 67

B. Period of Incubation

The period of time which elapsed between deposition of the eggs and hatching of the larvæ from them was obtained by daily examination of the cotyledons and secondary leaves in situ upon the seedlings by transmitted light with a low-power binocular microscope. Some leaflets in which larvæ were in process of hatching were removed, fixed in Carnoy's fluid, stained with borax carmine and mounted in Canada balsam for more detailed examination.

Records of the incubation period were obtained for a large proportion of the viable eggs laid by a single female fly during February; the seedlings in which the eggs had been laid were kept in a heated glasshouse in which night temperatures did not fall below 60° F.

During April, the period of incubation was obtained in similar manner for the majority of viable eggs laid by two females reared from eggs laid by the former fly, and a third obtained from a puparium collected on a commercial nursery: the plants containing eggs of these three flies were kept in an unheated glasshouse in which minimum night temperatures were in the region of 50° F. and over the whole period from April 8th to 27th fell below this for only 40 hours. Below a temperature of 50° F. it was assumed that development was not likely to proceed.

The records obtained are tabulated in Table I, and indicated that the average period of incubation was six days at temperatures usually obtaining in glasshouses in which tomato plants are raised.

TABLE I
PERIOD OF INCUBATION

Eggs			Period in Days		Temperature °F.			
No.	Laid	Hatched	Mean	Range	Mean	Mean Min.	Mean Max.	Range
57	4-14.II	9-21.II	5.6	4-6	69.7	64.3	81.6	62-89
40	8-18.IV	12-21.IV	6.2	5-8	67.1	52.5	94.5	43-104
100	8-18.IV	14-25.IV	6.3	5-8	66.8	51.9	95.1	
62	12-21.IV	19-27.IV	5.9	4-8	68.7	53.0	95.7	

C. Larval Life

The great majority of eggs laid by the flies were viable: some eggs which had been deposited amongst dense conglomerations of feeding pits failed to hatch, possibly through desiccation of the plant tissues immediately surrounding them.

When about to hatch, the larva was orientated with the anterior extremity, containing the mouth-hook, at the end of the egg furthest from the puncture made previously by the ovipositor of the fly. From an examination of stained preparations, it appeared that pressure exerted by the larva caused the eggshell to become distended longitudinally and eventually to split at its anterior end. The mouth-hook was then employed to break down the plant cells in its immediate proximity and the mine thus started was continued in a forward direction as a continuation of, and at a slight angle to, the walls of the egg-pit.

Owing to the flies having been confined within a limited space and provided only with a single plant of comparatively small size over given periods of time, the number of eggs deposited in the foliage of each plant was probably greater than it would have been under more natural conditions. When many larvæ hatched within a leaflet, they tended to tunnel into the stalks and thence into the main-stem of the plant.

The dimensions of the larva at the time of hatching were about 0.36 mm. in length, and 0.10 mm. in greatest diameter: the mouth-hook was feebly sclerotised and had an average length of 26μ (range 24μ - 28μ).

As far as it was possible to judge, the larvæ remained in the first instar for about four days. Shortly after the first moult, the second instar larva had a length of about 1.5 mm. and diameter of 0.25 mm.; the length of the mouth-hook, now very strongly sclerotised, was 52μ .

The duration of the second and third instar could not be determined owing to entry of the larvæ into the main-stem of the plant. By dissection of puparia, in which the larval mouth-parts were always embedded in the wall of the puparium, at its extreme anterior end, the length of the mouth-hook of the third instar larva was found to be about 72μ .

A very rough estimation indicated that the younger larvæ travelled a distance of about three-quarters of an inch in the course of 24 hours through the leaf tissues.

The total duration of the larval life from hatching of the egg to the time at which the larvæ vacated their mines in order to pupate was about 10 days.

Table II gives the duration of the larval life as recorded for some of the larvæ which hatched from eggs laid by each of the four females previously referred to (see Table I).

The mean minimum temperature recorded over the period during which the larvæ developed in February (in a heated glasshouse) was nearly 14° F. above that recorded while the larvæ mined in the plants in April and May (in an unheated glasshouse): between April 14th and May 10th temperatures fell below 50° F. for a total of 69 hours. during which development was assumed at least to have been considerably retarded. On the other hand, the position relating to the mean maximum temperatures over the respective periods was practically reversed, and it was concluded that this was responsible for the average period of development having remained constant.

TABLE II
DURATION OF LARVAL LIFE

Larvæ			Period in Days		Temperature °F.			
No.	Hatched	Vacated Mines	Mean	Range	Mean	Mean Min.	Mean Max.	Range
28	9-21.II	20.II-4.III	9.5	7-11	68.7	64.3	83.4	56-91
35	16-21.IV	25.IV-2.V	9.5	9-11	67.1	50.6	97.1	43-106
81	14-25.IV	25.IV-9.V	9.5	9-16				
59	19-27.IV	26.IV-10.V	8.9	7-13				

D. Pupation and Duration of the Pupal Instar

When full-fed, the larvæ tunnelled to the surface of the leaf or stem in which they had been mining, and cut a slit in it by means of the mouth-hook. Several larvæ were actually kept under observation while they perforated the stem or upper surface of a leaf, and it was noted that they remained within the mine for periods up to about an hour before they effected an emergence from it, and then fell to the ground in which they immediately buried themselves to a depth of about $\frac{1}{4}$ in. Only very rarely did a larva form its puparium on the leaf or stem of the plant.

The seven tomato plants in which 40 larvæ were known to have vacated their mines between February 23rd and March 3rd were cut down and the lamp-glasses which had been used to cover the plants were replaced over the soil in the pots so that subsequent emergence of flies from the puparia could be recorded. The pots were kept in an unheated glasshouse. Between April 2nd and May 3rd, 19 flies emerged (see Table III) and for reasons undetermined, no further flies were obtained after the latter date.

When the larvæ reared during April were about to vacate their mines, the pot-soil of each of the 16 plants containing them was covered with a disc of paper, cut so as to fit closely round the stem, and the pots were stood upon sheets of moist blotting-paper, from which the larvæ were collected daily, and allowed to pupate in soil or on moist cotton-wool contained in glass tubes covered with muslin: 186 puparia thus obtained between April 25th and May 10th were kept in the laboratory, where temperatures fluctuated to a much smaller degree than in the glasshouse in which the previous batch of puparia had been kept.

Between May 15th and 30th, there emerged from the puparia 104 flies, and between May 19th and June 6th 57 hymenopterous parasites. The 25 puparia which remained unemerged were dissected in September; eight each contained a dead almost fully-developed fly still within the pupal skin, seven each a pupa which had died at an early stage of development, and 10 each a dead larva which had not performed the ecdysis to the pupal instar.

Table III gives the duration of the pupal period as recorded for puparia reared respectively from eggs laid by a single fly during February, and by each of three flies in April and May, with mean temperatures which prevailed over the period of pupation.

TABLE III
DURATION OF PUPAL INSTAR. PUPARIA FROM LARVÆ REARED FROM THE EGG

Puparia			Period in Days		Temperature °F.			
No. and Sex	Formed	Emerged	Mean	Range	Mean	Mean Min.	Mean Max.	Range
{ 9 males 10 females	25.II-3.III 21.II-3.III	2.IV-1.V 5.IV-3.V	57.2 57.2	36-63 39-65	64.6	54.6	84.8	44-99
{ 10 males 13 females	25.IV-1.V 25.IV-1.V	16-20.V 16-18.V	19.6 19.5	19-21 18-21				
{ 26 males 25 females	25.IV-1.V 25.IV-9.V	15-23.V 15-30.V	19.7 19.4	17-22 19-22	63.3	61.0	65.1	60-70
{ 1 male 9 females	27.IV 27.IV-10.V	16.V 16-30.V	19.0	18-21				

The records obtained showed that the duration of the pupal period was the same for both sexes. Although the mean temperatures recorded differed by little more than 1° F., development within the puparium took on the average three times longer when the mean minimum temperature had been about 55° F. (with some 120 hours below 50° F.) than it did when the mean minimum had been 6° F. higher (with an absolute minimum of 60° F.).

Observations upon puparia obtained from larvæ collected in glasshouses showed, however, that differences in temperature were not the cause of the pupal period being prolonged or attenuated.

Thirty-one puparia obtained during February were kept in a heated glasshouse in which the temperature fell to 48° F. only for four hours over the whole period (see Table IV). The average period of development for the 14 flies which subsequently emerged from them was 51 days; from three puparia hymenopterous parasites emerged respectively 18, 102 and 112 days after their formation. When dissected in June, 18 weeks after they had been formed, four unemerged puparia were found to contain each a dead male fly still enveloped by the pupal skin, four each a dead pupa and two each a larva which had died before ecdysis to the pupal instar; the remaining four puparia each contained a fully-developed living larva of an hymenopterous parasite.

Forty-nine puparia obtained early in June were kept in the laboratory in which the temperature did not fall at any time below 60° F. (see Table IV). The average period of development within the puparium for 28 flies which subsequently emerged was 18 days, and a hymenopterous parasite emerged from one puparium 20 days after its formation. Of the 20 unemerged puparia dissected some months later, five contained dead flies (three males and two females) still enveloped in the pupal skin, six dead pupæ, and in nine the larvæ had died before ecdysis to the pupal instar.

Table IV gives the duration of the pupal period as recorded for these puparia:—

TABLE IV
DURATION OF THE PUPAL INSTAR. PUPARIA FROM LARVÆ COLLECTED IN GLASSHOUSES

Puparia			Period in Days		Temperature °F.			
No. and Sex	Formed	Emerged	Mean	Range	Mean	Mean Min.	Mean Max.	Range
{ 7 males 7 females	8.II-16.II	12.III-7.IV	40.6	31-52	68.2	61.1	85.8	48-104
	11.II-16.II	26.III-26.IV	61.6	43-69				
{ 18 males 10 females	1-3.VI	17-23.VI	17.5	16-22	—	—	—	60-70
	1-3.VI	16-22.VI	17.7	15-21				

The difference between the period of development for males and females within the puparia obtained in February is not considered to have been significant.

Comparison of the records set out in Tables III and IV shows that development to maturity took, on the average, three times longer in puparia formed in February than it did in puparia formed from April to June, irrespective of the temperatures which prevailed during the period of pupation.

Under whatever conditions in respect of moisture the puparia were kept, whether in moist soil or upon dry wool contained in open glass tubes, in a large proportion (from 20 to 50 per cent.) the insect failed to complete its development, and sometimes had died within the pupal skin when nearly mature.

From the records obtained, it was concluded that development within the puparia obtained in February was prolonged as a result either of the larva or more probably the pupa having entered a state of diapause for a more or less definite period of time. The factors inducing this state were not ascertained, but it is suggestive that during the period over which the larvæ developed in February (see Table II) the total hours of sunlight recorded was 87.1 (daily average 3.6 hours), as compared with 220.9 (daily average 8.2 hours) over the period of development in April and May.

It was noteworthy that most of the Hymenopterous parasites contained in parasitised puparia of the fly collected in February had not even arrived at their own pupal instar 18 weeks after these puparia had been formed, and had evidently entered a very prolonged period of diapause after having eaten out the contents of the puparium and become full-fed.

In parasited puparia obtained from April to June, on the other hand, the parasites completed their development and emerged as adults in from 16 to 30 days after formation of the puparia, so that, as in the case of their host, no state of diapause was entered at that time of the year.

II. Injury caused by the Larvæ

In the case of young tomato seedlings before the time of potting up, destruction by the larvæ of the tissues in the cotyledons prevents normal development of the plants and may bring about their total collapse. After the seedlings have been potted and the cotyledons normally begin to lose their function, injury caused by larvæ mining in the secondary leaves and main-stem is not so severe as might be expected.

Only one or two of the 25 plants in "60" size pots employed for the purpose of recording the duration of the larval life were damaged beyond recovery, though in the majority of them some 10 to 15 larvæ completed their development—a number at least equal to that which would be likely to occur in severe infestations during the earlier months of the year.

Of three plants in which respectively 4, 14, and 16 larvæ completed their development, the lowest two, and in one of them the lowest three, secondary leaves had shrivelled by the time the larvæ had vacated their mines between February 21st and March 4th. By March 16th the plants had attained a height and leaf span practically equal to those of uninfected plants of the same age. Photographs (Figs. 1, A and B) taken of these plants respectively on February 22nd and March 16th serve to illustrate the initial injury caused and the degree of recovery made by the plants in the course of three weeks.

III. Effects of Insecticides upon the Larvæ and Adult Flies

(a) APPLICATION OF POWDERS CONTAINING DDT AND BHC TO THE SOIL SURFACE

Experiments on the lines carried out previously (Rep. Exp. Sta., Cheshunt, 1948, pp. 50-51) to determine if the larvæ would succumb when burying themselves in soil dusted with the powders, or if the adult flies would be killed on finding their way to the surface after emergence from the puparia, were repeated under more favourable conditions of temperature. The shallow dishes which contained the soil were kept over wet sand in large glass vessels covered with muslin. These conditions, however, were not effective in inducing a higher proportion of insects to emerge from the puparia. Ten larvæ, obtained in February and dropped onto the soil surface immediately after 5 per cent. DDT powder had been applied to it, buried themselves and formed puparia normally; both pupæ of the flies and hymenopterous parasites contained in the puparia subsequently completed their development.

In a similar experiment with BHC powder, six out of 10 larvæ formed their puparia normally: in three of the latter the flies developed to maturity and in the remainder the larvæ at least performed the ecdysis to the pupal instar.

Shortly before the insects were due to emerge from puparia obtained in June, the surface of soil in which the puparia had been buried to a depth of about one-eighth inch, was dusted, in one case with DDT and in the other with BHC powder.

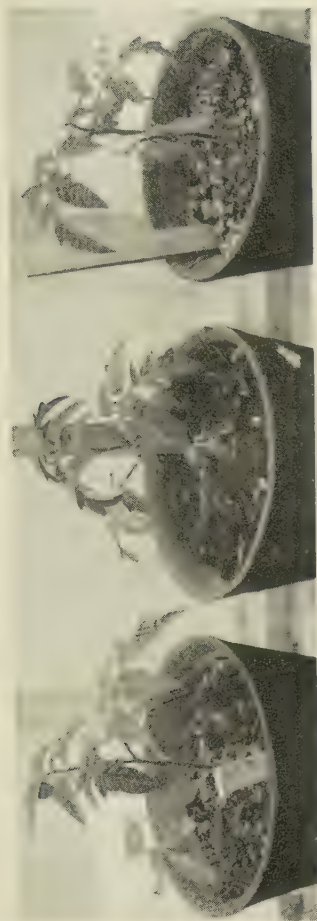
The few flies and hymenopterous parasites which emerged from the puparia within a period of four days after application of the dusts, died shortly after they had come into contact with either of them. It was noted that all the insects had a considerable amount of the powders adhering to their bodies.

(b) BHC POWDER APPLIED TO INFESTED TOMATO SEEDLINGS

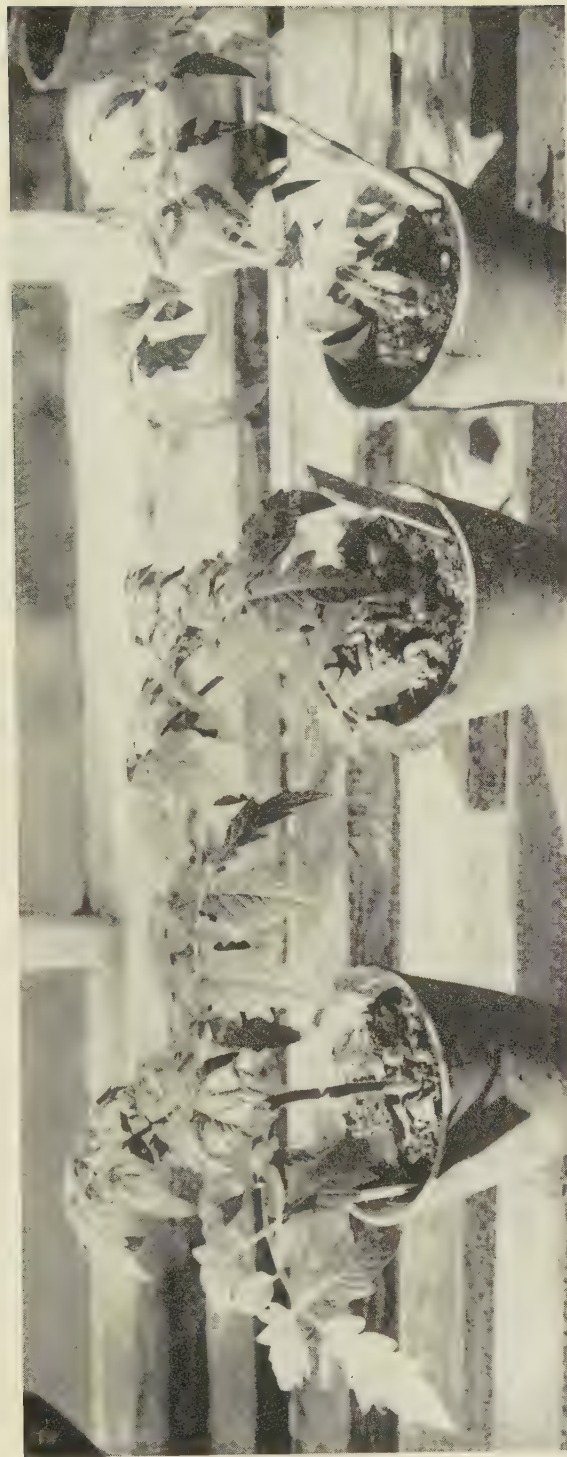
During February a 5 per cent. BHC powder, containing 0.65 per cent. γ -isomer, was applied to the foliage of tomato seedlings in "60" size pots, which were kept in a glasshouse in which temperatures ranged from 44° F. to 83° F. In the case of four plants, with an average height of 1½ ins. larval mines were present only in one or both cotyledons of each at the time of application. Two days after, four dead second instar larvæ were located in the mines, which had not been extended to an appreciable extent. Subsequently no larval mines made their appearance in the secondary leaves.

By the third day, the secondary leaves of the plants had become inwardly curled, and by the ninth day after application brown patches had made their appearance upon them. Subsequently the leaves became severely distorted, and after five weeks the plants had attained a height of only 4 ins. In two similar plants to which no powder was applied, the larva contained in each extended its mine into the secondary leaves after two days, and in the course of five weeks the seedlings attained a height of 6 ins.

From the larval mines in the secondary leaves or stems of five plants with an average height of 3 ins., five dead larvæ (in the second or third instar) were located between the fifth and seventh day after application of the powder: one larva was still active on the fifth day, but had succumbed by



A. Plants in which (left to right) 14, 16, and 4 larvæ are feeding.



B. The same plants three weeks later.



FIG. 2.—To illustrate effect of BHC powder on growth of tomato plants. $\times \frac{1}{4}$

A. Unaffected control plant.

B. Plant to which powder had been applied seven weeks previously

the ninth day. No puparia were subsequently obtained from the soil in the pots. The plants developed initial symptoms of injury similar to those recorded previously, and in the course of three weeks yellow speckles made their appearance upon the distorted leaves. Seven weeks after application of the powder the plants, one of which is shown in Fig. 2B, had attained an average height of 6 ins. In each of two similarly infested plants, which were not dusted with the powder, the larvæ completed their development and pupated in the soil of the pots: in the course of seven weeks the plants, one of which is shown in Fig 2A, had attained a height of over 8 ins.

It was concluded from these experiments that a 5 per cent. BHC powder was effective in killing the larvæ in their mines, but could not be used upon young plants without risk of severe injury to them.

(c) BHC EMULSION APPLIED AS A SPRAY TO TOMATO SEEDLINGS

It was considered probable that emulsified BHC applied as a spray would be effective in destroying the larvæ in their mines and less likely to cause injury to the plants than powders containing the compound in a solid state. Messrs. Plant Protection Ltd. kindly provided for the purpose an emulsion which contained 32 per cent. BHC. For application this was diluted at the rate of one part in 400 parts water, so that the spray as applied contained 0.08 per cent. BHC (and 0.01 per cent. γ -isomer of the compound). Plants with average height respectively of $\frac{3}{4}$, 2, and 5 ins. sprayed with the diluted emulsion in February subsequently showed no signs of any injury, and in the course of three weeks attained an average height respectively of 3, $4\frac{1}{2}$ and $9\frac{1}{2}$ ins., which did not differ from those of unsprayed plants used as controls. No plants containing larval mines were available at the time, but the mines in leaves collected from infested plants, to which the spray had been applied three days previously on a commercial nursery, contained no living larvæ. As is usual in such random samples, the majority of the mines had been vacated by the full-fed larvæ prior to application of the spray, but the few larvæ, some young and others nearly ready to pupate, still present in them were dead.

These experiments appeared to indicate that a spray containing BHC applied to seedlings in the "pot" stage might be of considerable value for the purpose of destroying the larvæ in their mines, and so of reducing the risk of future infestation from flies which otherwise would emerge from puparia contained in the soil round the roots at the time of planting out in the ground.

(d) EFFECT OF NICOTINE UPON THE LARVAE

In February, infested tomato seedlings in "60" pots were dipped in 0.1 per cent. nicotine solution containing 0.01 per cent. Agral N as a wetting agent.

In plants with average height of $1\frac{1}{2}$ ins. and larval mines confined to the cotyledons, a dead larva was located in each mine on the third day after treatment, and subsequently no mines made their appearance in the secondary leaves, nor were puparia found in the soil of the pots. There was no injury to the foliage, and in the course of five weeks the plants attained an average height of $6\frac{1}{2}$ ins., equal to that of similarly infested but untreated plants in which the larval mines had been extended into the secondary leaves: puparia were subsequently recovered from the soil of the pots in which these control plants were grown.

In plants with average height of 3 ins. and larval mines present in the secondary leaves, younger larvæ located in the mines five days after treatment were dead, but older ones had extended their mines and were active.

There was some slight scorching of the edges of the leaves and seven weeks after treatment the plants had attained an average height of $7\frac{1}{2}$ ins., as compared with one of over 8 ins. by similarly infested but untreated plants.

It was concluded that the dipping of young pot plants in nicotine solution was of little practical value as a means of destroying the larvæ in their mines.

(e) EFFECT OF PYRETHRUM POWDER UPON THE ADULT FLIES

A male fly to the body of which a quantity of pyrethrum powder was adhering after it had been in contact with a lightly dusted surface for 30 seconds made short rapid flights and after five minutes lay ventral surface upwards. Twitchings of the legs and intermittent spasms continued during the following six minutes, and after five hours all movement had ceased. No recovery was made.

In similar circumstances, a female fly which had emerged from the puparium on the previous day gradually became paralysed and in 10 minutes lay ventral surface upwards with twitching movements of the legs and repeated extrusion of the ovipositor, but subsequently made a gradual and complete recovery. Another newly-emerged female, which had wandered over a tomato leaflet

dusted with the powder for two minutes and was then removed to a clean leaf, became paralysed in the same manner in five minutes and its movements remained unco-ordinated for a period of more than three hours, after which it made a complete recovery.

No trace of six male and five female flies could be found two days after they had been liberated in cages containing tomato seedlings to which the powder had been applied.

In one cage containing 35 untreated and 38 dusted plants, 1 to 2 ins. in height in a seed-box, three female flies made feeding-pits upon eight cotyledons of the former and none were made upon the cotyledons of the latter seedlings: no eggs were deposited.

In another containing one untreated and three dusted pot plants, each 4 ins. in height and with five secondary leaves expanded, the percentage of leaflets with feeding-pits made by two females upon the former plant was 26, and upon the latter plants seven. One egg was deposited in a leaflet of one of the dusted plants and the larva which hatched from it subsequently completed its development and pupated.

As a result of these observations which were recorded in June, it was considered probable that applications of pyrethrum powder would, without any risk of injury to them, at least afford some measure of protection to very young seedlings from flies which might emerge from puparia contained in the soil of propagating houses. The lethal action of the powder upon the flies was very uncertain, but its mechanical action in preventing the females from puncturing the leaves with their ovipositor was marked.

Its use could be recommended only over limited areas of plants because application at four-day intervals during the whole period of propagation would be necessary in order to afford adequate protection to the seedlings.

IV. Reaction of the Adult Flies to Baits

Flies of both sexes had been observed to feed upon syrup and the possibility of employing traps for them presented itself. Very few flies, however, were caught in jars containing sweetened baits and kept in cages in which the insects also had access to tomato plants: none were trapped in similar jars placed on staging of a propagating house in which adult flies were abundant.

After having fed upon a sweetened bait containing 1 per cent. sodium fluoride and upon another, employed for destroying house-flies, containing glucose and formaldehyde, they succumbed after a few hours.

The flies attempted to feed upon, but were not especially attracted to, a 10 per cent. sugar solution containing 5 per cent. amyl alcohol, used as an attractant for some other insects: on coming into contact with small pieces or rubber sponge impregnated with the mixture, they died in the course of a few minutes.

2. Studies upon Thysanoptera

By E. R. SPEYER and W. J. PARR

(1.) Genus *Aeolothrips*

Further study of the British species makes necessary the following corrections, with additional information, in the notes published in 1948 (Rep. Exp. Sta., Cheshunt, pp. 53-57).

(a) Reference to Haliday's publication of 1836, in which the genus is divided into the two sub-genera *Aeolothrips* and *Coleothrips*, shows that the type species should be *Ae. albicinctus* Hal., and not *Ae. fasciatus* L. Although males of *albicinctus* are always and females are usually brachypterous, the raising of Haliday's sub-genera to the status of genera is not considered to be justifiable, since macropterous forms of the female are now known to exist.

(b) By kind permission of the British Museum (Natural History) authorities, mounts for microscopic examination were made of the specimens labelled "*Coleothrips fasciata* L." in the collection assembled by F. Walker and referred to in his catalogue of 1852. The insects were glued to cards or pins, and it is reasonable to suppose that they were collected by Haliday in or before the year 1836. After mounting, they were readily determinable, and comprised one female *Ae. fasciatus* L., one male and three female *Ae. tenuicornis* Bagn.

(c) *Ae. fasciatus* L.—Measurements of the fourth antennal joint in a series of specimens collected from various plants in different localities were obtained. The average ratio of length to diameter in 13 males was 3.77 (range 3.2-4.0), and in 39 females 3.60 (range 2.6-4.3); the joint was therefore not more than four and a half times as long as the diameter. In all the specimens the fourth antennal

joint was distinctly longer than the fifth joint; in the males the raised processes upon the fourth and fifth abdominal tergites were conspicuous, and the "interstitial" setæ did not project beyond the apex of the claspers (*vide* Priesner 1948, p. 325, Fig. 10).

As far as it was possible to ascertain, no British specimens in the British Museum, Dr. C. B. Williams', or other collections, possessed the characteristics attributed to "*fasciatus* L." by Priesner (1948).

It was concluded that the species represented in Britain conformed with the type specimen of *Ae. intermedius* Bagn., and being (with the possible exception of *Ae. tenuicornis* Bagn.) the most abundant one in Northern Europe, was most probably that to which Linnæus gave the name "*fasciata*."

(d) *Ae. erica* Bagnall.—Males of the species possess a pair of raised sclerotised processes upon the eighth as well as upon each of the fourth to sixth abdominal tergites; this affords an additional characteristic for the separation of males from those of other species cited in the key (*loc. cit.* p. 56).

(e) *Ae. tenuicornis* Bagnall.—Large numbers were collected in the flowers of cultivated poppy at Cheshunt in July, in absence of other species of the genus; amongst them were 12 pairs in coitu, of which both sexes possessed the characteristics set out for this species in Table IV (*loc. cit.* p. 56). Some larvæ collected from these flowers showed, as far as could be ascertained, no characteristic by which they could be separated from larvæ either of *Ae. fasciatus* L. or of *Ae. erica* Bagn.

In a very long series of British females, the median setæ upon the posterior margin of the seventh abdominal sternite were 35–40 μ apart, and thus more closely approximate to one another than was found to be the case in females of *Ae. fasciatus* L. Where some doubt may arise as to whether the costal vein round the apex of the forewing is shaded or clear, the proximity of these setæ affords a reliable means of separating females of *tenuicornis* from those of *fasciatus*, in which the setæ were 75 to 120 μ apart, but not from those of *Ae. erica* in which they ranged from 40 to 130 μ apart. (See Priesner 1948, p. 319, Figs. 1 and 3.)

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 SPEYER, E. R., and PARR, W. J., Rep. Exp. Res. Sta., Cheshunt, pp. 53–57, 1948.
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3. INSECTICIDE INVESTIGATIONS

By W. H. READ

(A) Laboratory Investigations

Acaricides

The investigation of the ovicidal properties of a large number of compounds on the eggs of the glasshouse red spider mite (*Tetranychus telarius* L.) has been continued. The method of biological assay described in the 1948 Report¹ was employed in these tests. Particular care was taken in selecting test material from young leaves recently infested with mites and in addition leaf discs were rejected on which any eggs were abnormal in appearance due to discolouration or shrinkage. In spite of these precautions considerable variations in the natural mortality of untreated eggs were recorded which did not appear to be due to accumulation of non-viable eggs on the test material. Allowance for day-to-day variation in the resistance of eggs to the action of ovicides was made by comparing each substance with a common ovicide, usually azobenzene.

The most outstanding ovicide amongst those examined was di-(*p*-chlorophenyl) methyl carbinol which, was more toxic than azobenzene at the 95 per cent. kill level. Laboratory tests with a commercial preparation consisting of a 25 per cent. emulsified solution of the compound gave toxicity figures as an ovicide against red spider eggs much higher than might be expected from its di-(*p*-chlorophenyl) methyl carbinol content. Although spraying tests with plants suggest that the insecticidal efficiency of the compound in the preparation may be enhanced by other ingredients, the method of laboratory assaying by the dipping technique appeared to give abnormally high toxicity due to preferential wetting of the eggs and leaf discs by the oil phase of the diluted emulsion. The dipping technique cannot be considered satisfactory for the comparison of emulsions with suspended solids or solutions. Diphenyl carbinol had little ovicidal action as had di-(*p*-chlorophenyl) *n*-butyl carbinol, *p,p'*-dichlorobenzophenone, *p,p'*-dichloro-diphenyl and di-(*p*-chlorophenoxy) methane.

Small-scale spraying trials were made against red spider mites with the commercial preparations, stated to contain 25 per cent. of di-(*p*-chlorophenyl) methyl carbinol. In these tests 99.5 per cent. control of all stages of the mite was obtained by a single application of the product at a dilution of 1 in 2,000 (0.0125 per cent. di-(*p*-chlorophenyl) methyl carbinol. Tomatoes, approximately 3 ft. high, were uninjured by this concentration but cucumber foliage was mottled and distorted and the growth rate of the plant reduced, the injury resembling that caused by DDT, a compound somewhat similar in molecular structure to di-(*p*-chlorophenyl) methyl carbinol. Tomatoes were slightly "hardened" by the preparation at a dilution of 1 in 1,000.

Methane sulphonyl fluoride was tried later in the season as a fumigant using the green peach aphid (*Myzus persicae*) and red spider mite as test insects. In laboratory trials 100 per cent. kill of active stages of the mite was obtained by exposure for four hours to the equivalent of 3.5 grams methane sulphonyl fluoride per 1,000 cu. ft. At this concentration 33 per cent. control of resting stages was obtained but the fumigant had no effect upon eggs. Adult and immature aphids were also killed.

Fungicides

Studies on the fungicidal action of a series of organic compounds has been commenced with the two-fold object of investigating the effect of constitution on fungicidal properties and obtaining a substance which can be used to control diseases of glasshouse plants.

The *in vitro* tests are being made by a "test-tube dilution" method.

(B) Glasshouse Experiments

By W. H. READ

(I) Systemic insecticides

Two of the group of compounds termed systemic insecticides by virtue of their ability to be absorbed and translocated by plants and render the tissues poisonous to certain insects, have been employed in glasshouse trials. The two substances tested were bis-(bis-dimethylamino) phosphonous anhydride (I) and bis-dimethylamino fluorophosphine oxide (II). These substances are highly poisonous, particularly (II) consequently it appears unlikely that they will be applicable to glasshouse food crops but (I) may be of value for the control of pests on flower crops.

Absorption into plants can take place either through the roots or through the foliage.

Soil Application

Preliminary experiments were made to determine whether control of aphids and red spider could be obtained by application of these substances to the soil without injury to plants. In view of the difficulty of obtaining satisfactory control of red spider and aphids (*Myzus persicae* on carnations, together with the possible advantages which might be gained by the elimination of sprays on these plants, much of this preliminary work has been done with young carnations grown in pots.

The following is a summary of the results obtained in tests in which the insecticides were applied to the soil of pot plants during hot, bright weather in June. The plants were grown in a well-ventilated glasshouse but no precautions were taken to eliminate fumigant effects.

(I) BIS (BIS-DIMETHYLAMINO) PHOSPHONOUS ANHYDRIDE

Host Plant	Amount per 100 grams soil (ml.)	Time taken (days) to give 100% Control		Phyto Toxicity
		Aphids*	Red-spider*	
Carnation ...	0.05	10	5	None
" ...	0.025	> 14	10	"
" ...	0.012	> 14	14	"
Cucumber ...	0.035	—	3	None—14 days
" ...	0.01	—	7	"
Tomato ...	0.05	—	3	" "
" ...	0.025	—	3	" "

(II) BIS-DIMETHYLAMINO FLUOROPHOSPHINE OXIDE

Host Plant	Amount per 100 grams soil (ml.)	Time taken (days) to give 100% Control		Phyto Toxicity
		Aphids*	Red-spider*	
Carnation ...	0.05	2	2	Plant killed
" ...	0.025	2	2	Very severe
" ...	0.012	4	2	Severe
" ...	0.006	6	6	"
" ...	0.003	14	6	Very slight
Cucumber ...	0.05	—	2	Very severe
" ...	0.025	—	2	"
" ...	0.012	—	2	Severe

These tests show that the control obtained by watering plants with solutions of (II) is better than that obtained by watering with equal amounts of (I) but that (II) is much more phytotoxic. The period during which plants grown in soil treated with (I) remained toxic to aphids and red spider showed very considerable variation between plants treated with the same quantities of the insecticide. Furthermore, red spider mite survived when placed on the old leaves whereas those on the young leaves failed to do so. Aphids, however, migrated to the young leaves and tips of the growing shoots and consequently were killed over a longer period. The period of protection against aphids under the conditions of these tests was about four weeks where (I) was used at the rate of 0.05 ml. per 100 grams of soil.

The bis-dimethylamino fluorophosphine oxide has also much greater hazards attached to its use and no further work on its practical application was carried out.

Tests with (I) on carnations under the low temperature conditions during November showed that the uptake of the insecticide was so slow that 0.05 ml./100 grams soil did not control aphids, within a period of three weeks.

Spray application

In these tests young carnation plants infested with *Myzus persicae* were sprayed with solutions of bis-(bis-dimethylamino) phosphonous anhydride containing 0 per cent., 0.1 per cent. and 0.2 per cent. Agral 2 (a proprietary wetting agent). Examination of the plants after five days showed that 100 per cent. kill of aphids was obtained under the conditions of the test by the use of 0.1 per cent. (I) in combination with 0.2 per cent. Agral L; 0.2 per cent. (I) in combination with 0.1 Agral L. and by 0.3 per cent. (I) in the absence of any wetting agent.

Owing to the injury inflicted by the aphids of these plants the degree of marking of the foliage by the different sprays could not be estimated.

Summary

These exploratory tests made with the object of finding if systemic insecticides might prove of value for the control of pests of flower crops, in particular carnations, have shown that bis-(bis-dimethylamino) phosphonous anhydride is very promising for the control of aphids and red spider on carnations and further work is justified.

Spraying the plants with solutions gives a more rapid control with economy of insecticide as compared with the application of the insecticide to the soil.

No information has been obtained regarding the effect of repeated applications on the growth and flower formation of carnations or the effect of cultural and growth conditions on the period of protection afforded. Results obtained with carnations, *Myzus persicae* and red spider mites afford little information as to the reactions which will be obtained on other plants and insects.

(2) Red-spider control

Further experiments on the control of the glasshouse red spider mite (*Tetranychus telarius* L.) with parathion (*p*-nitrophenyl diethyl thiophosphate) and azobenzene-parathion mixtures have been made.

Work at Cheshunt¹ showed that about 13 per cent. of the active stages of the red spider survived for five days or more after treatment with 8 grams of azobenzene per 1,000 cu. ft. dispersed as an "aerosol." Whilst some of these might have been in a resting stage when treated with azobenzene the majority of the mites surviving were adult females which were capable of laying viable eggs and consequently repeated applications were necessary to obtain satisfactory control.

A dosage of 8 grams per 1,000 cu. ft. has been found too near the limit of safety for commercial application on some crops, particularly cucumbers, and a dosage of 6 grams per 1,000 cu. ft. is more frequently used.

Where frequent applications of this amount of azobenzene were given, either in "aerosol" or "smoke" form, the control of red spider obtained was initially superior to that given by the use of white-oil emulsions under commercial conditions and was obtained with economy of labour. Other methods of control have been necessitated by tolerance to azobenzene now shown by the mites, probably resulting from the destruction by azobenzene of the less-resistant mites and the reproduction of the more resistant ones. This has made it necessary to find an alternative or modified method of control.

Continuation of the trials with HETP (hexaethyltetraphosphate) and more recently with TEPP (tetraethyl pyrophosphate) have shown that these are not effective when dispersed as aerosols by the compressed-air-spraying method,¹ either for the control of active stages when employed on their own or for increasing the action of azobenzene on active stages when employed in azobenzene-TEPP (or HETP) mixtures.

Parathion (diethyl *p*-nitrophenyl thiophosphate or E605) was shown in 1948² to be very effective against active stages of red spider in that 0.3 gram/1,000 cu. ft. of commercial parathion gave complete control of active stages of the mite and the combination of 4 grams azobenzene and 0.3 grams parathion/1,000 cu. ft. gave a better control than 6.0 grams/1,000 cu. ft. of azobenzene. Further trials with parathion* and azobenzene-parathion mixtures have been made in glasshouses. The parathion and parathion-azobenzene mixtures were dissolved in acetone and applied by the compressed air-spraying method.

A respirator was worn when applying them and it should be emphasised that although this method was adopted for experimental purposes the extremely poisonous nature of parathion renders this method quite unsuitable for the commercial application of parathion. Unfortunately, the poisonous nature of parathion made it impossible to conduct replicated or comparative trials in the main blocks of glasshouses at the Research Station.

Pot plants were employed in most of these tests and after treatment data regarding the results was obtained as follows:—

For assessment of the true ovicidal action, leaf discs having on them eggs of the mite were removed from treated plants, gummed to slides and all non-egg stages removed in the same manner as in the laboratory ovicide tests.

The kill of adult female mites was usually determined by removing all other stages from leaflets of treated plants, the stalks of the leaflets were banded with vaseline to prevent migration thereto by other mites and the number of mites showing movement 48 hours after treatment were recorded as survivors. In some tests, however, adult mites were carefully transferred from the treated plants to untreated detached leaves placed in closed petri-dishes.

The survival of larvæ emerging from treated eggs or of active stages from resting stages developing after treatment was determined by keeping the plants for 15 to 20 days and then examining them for the presence of living mites. Insufficient time was available for more precise determination of the period during which residual action was sufficient to kill newly-hatched larvæ. For this reason and also on account of the tedious work and eye-strain involved in counting eggs and mites, control counts were not made with material taken before treatment.

The results of the trials are summarised in the table. They confirm that the addition of parathion to azobenzene yields a very much better control of red spider than is obtained by azobenzene alone. The quantity of parathion required to obtain a 100 per cent. control of all stages by the killing of larvæ hatching from eggs or active stages developing from resting stages, is high.

Parathion is highly poisonous not only when taken orally but also when absorbed through the skin by contact with extremely small residues on treated foliage, etc. There have been many cases of injury to workers in other countries due to the absorption of parathion from residues on sprayed foliage. For this reason the use of quantities of parathion sufficient to give residual toxicity to insects is most undesirable with most glasshouse crops.

Although compressed-air-spraying was employed for the production of aerosols in this experimental work the procedure is far too dangerous for use on commercial nurseries.

Parathion can be dispersed as a smoke and a number of proprietary smoke generators, tested in glasshouse trials, have given very satisfactory results in the control of insect pests. More information regarding the hazards accompanying the use of parathion smokes is required, however, before their use can be recommended.

No. of Trial	Dosage in grams per per 1,000 cu. ft.		Host Plants	Eggs % Kill at 14 Days	Adult Females % kill at 48 Hours	% Emergence of Mites from Resting Stages	Survival of Larvæ from Eggs and/or Mites from Resting Stages—15-20 days
	Azo-benzene	Parathion (80%)					
1	8.0	0.3	Tomatoes	99.0	98	7	Some survivors.
2	6.0	0.3	"	97.5	100	11	" "
3	4.0	0.3	"	98.5	97	17	Larvæ hatching three days after treatment survive.
4	6.0	0.3	Cucumbers	96.4	100	24	" " "
5	4.0	0.3	"	99.0	100	4	" " "
6	6.0	0.5	Tomatoes	97.0	98	20	" " "
7	—	2.0	Cucumbers	—	100	—	None.
8	—	1.0	Carnations	—	100	—	Very few survivors.
9	—	1.0	Tomatoes	—	100	52	Larvæ hatching five days after treatment, survived.
10	—	0.5	"	25.0	100	42	Many survivors.
11	4.0	0.5	Carnations	100.0	83	—	Adults survived in blossoms.
12	—	2.0	Tomatoes	—	100	—	None.
13	4.0	0.5	Cucumbers	100.0	100	5	Some survivors.
14	4.0	0.25	Tomatoes	95.0	81	—	Many survivors.

REFERENCES

¹ Rep. Exp. Res. Sta., Cheshunt, 1947, 33, 66—88.

² Rep. Exp. Res. Sta., Cheshunt, 1948, 34, 59—60.

* Approximately 80 per cent., diethyl *p*-nitrophenyl thiophosphate.



IV. CHEMICAL PROBLEMS

I. NITRIFICATION STUDIES

By G. W. WINSOR

Investigation of the decomposition of nitrogenous materials in the soil has been continued. The formation of ammonia and nitrate from amino-acids and related compounds has been studied in order to gain information concerning intermediate stages in the decomposition of proteins. Seven amino-acids have so far been used in these experiments, namely, glycine, alanine, aspartic acid, glutamic acid, arginine, tyrosine and cystine. All were readily deaminated and nitrified with the exception of cystine. For the amino-acids not containing sulphur, maximum values for ammonia were found within two to three days, after which they fell rapidly as nitrification proceeded. In the case of cystine, ammonia formation was somewhat slower and reached a maximum in 28 days. Nitrification was inhibited markedly by cystine or its decomposition products, and denitrification occurred in the first two weeks. At lower concentrations inhibition by cystine was less marked. It is of interest that values for the cystine content of horn varying from 6.8 to 8.3 per cent. are found in the literature. Hippuric acid was also found to retard nitrification.

The maximum values for both ammonia and nitrate formed in the soil from glycine ($C/N=1.71$) and from two treatments with glycine and glucose ($C/N=4.7$ and 7.7) were inversely related to the C/N ratio. Comparison of results for different amino-acids having C/N ratios from 1.29 to 7.71 suggested a similar relationship. In a maiden soil alanine was preferred as a substrate for nitrification to a mixture of ammonium sulphate and glucose of the same C/N ratio.

Analyses for ammonia after 24 hours showed considerably more rapid deamination of aspartic and glutamic acids than of the simpler glycine and alanine, though all four amino-acids reached their maxima in 48 hours. Further evidence of the ready deamination of amino-acids in the soil was gained by following the rapid loss of glycine colorimetrically using Folin's reagent.

Four commercial samples of mixed hoof and horn, one each of hoof and of horn alone, and three of calcined horn were compared on the basis of nitrate formation in the soil. It was found that all the horn samples were more slowly nitrified than mixed hoof and horn or hoof alone. The influence of alginates on nitrification has been tested in two soils. In a maiden soil nitrate formation from di-ammonium hydrogen phosphate was markedly reduced by alginic acid and its calcium salt throughout a period of five and a half weeks. Sodium alginate showed a slight stimulating effect in the first week but subsequently reduced nitrate formation. The nitrate levels of the original soil and of soil to which potassium nitrate had been added were both somewhat reduced by sodium alginate. Similar results for the effect of sodium and calcium alginates on the nitrification of di-ammonium hydrogen phosphate were obtained with a market garden soil, but in this case alginic acid showed no adverse effect on nitrate formation in the first two weeks (<60 per cent. nitrified).

In a tomato house soil which had been under cultivation for 20 years ($pH\ 7.5$) ammonium sulphate and di-ammonium hydrogen phosphate were more readily nitrified than the amino-acids glycine and tyrosine. In another soil, however, known to be rather infertile ($pH\ 6.0$) the positions of ammonium phosphate and glycine were reversed. A second batch of this latter soil, taken from the same nursery heap two months later, showed considerably greater biological activity as judged by rate of nitrification, and differences of rate for the various sources of nitrogen were small.

It was again shown for hoof and horn and dried blood that the percentage conversion to nitrate at any stage of the decomposition did not vary greatly with level of application, whereas with ammonium sulphate and glycine the percentage nitrified at all stages of active decomposition was inversely related to level of application. An explanation of this result can be given on the basis of ammonia production; where the percentage nitrified differed with level of application, ammonia was present in the soil. With hoof and horn and dried blood the ammonia values were relatively low, showing that the capacity of the soil to convert ammonia to nitrate was not greatly exceeded at any time. With ammonium sulphate and glycine, however, high concentrations of ammonia were present, indicating that the nitrifying capacity of the soil was being exceeded. At some stages in the experiment the actual quantities of nitrate formed, as distinct from the percentages, were almost independent of the level of application of ammonium sulphate or glycine.

It was previously reported that the sample of bone meal used in our experiments was very readily nitrified. A further sample supplied as bone meal was later found to be very slowly nitrified.

even after milling to a fine powder. The original material showed 43 per cent. conversion to nitrate in seven days as compared with 4 per cent. for the new sample. The nitrogen content of the original bone meal was 3.3 per cent. in 1946, but had fallen to 2.68 per cent. by 1949. On distillation with magnesia both in 1947 and 1949 it yielded 8 per cent. of the nitrogen as ammonia, whereas the newer sample was practically stable. The rapid nitrification of the older sample of bone meal may thus be attributed to a partial decomposition in storage. The results show the wide variations which exist between different samples of a nitrogenous fertiliser.

2. STEAM STERILIZATION STUDIES

By O. OWEN and J. N. DAVIES

Investigation of the effects of steam sterilization on ammonification and nitrification in soil has been continued.

The changes in four soils have been studied. Of these, three had been steamed repeatedly during a number of years; the fourth was a maiden loam which had not been steamed before and had not carried a glasshouse crop. In all cases steaming increased ammonia production. The extent and duration of this production varies in the different soils. In the maiden loam the ammonia level reached the high value of 250 p.p.m., while in the other soils the value rarely exceeded 50 p.p.m. In all the soils ammonification increases gradually at first but quickly accelerates to a maximum which eventually falls. In contrast the ammonia in the unsteamed soils remains at a consistently low level.

Meanwhile steaming has no pronounced effect on the nitrate level, which remains steady or falls slightly until just before the ammonia concentration starts to fall. Nitrification then starts and proceeds rapidly.

It so happens that the nitrogen and organic carbon contents of the maiden loam were approximately the same as those of one of the old soils which had been steamed before and the results for these two soils suggest that subsequent steamings do not cause such decided effects on ammonification as does the first.

This view is supported by the fact that at the end of one hundred days when the ammonia level in the steamed maiden loam was almost the same as that in the unsteamed soil re-steaming only caused an ammonia peak of about 70 p.p.m. as compared with a peak of about 230 p.p.m. after the first steaming.

The effect of re-steaming the maiden loam when the ammonia level was at a maximum has also been examined. Ammonia was maintained at a higher level for a longer period than in the same soil which had been steamed originally. The eventual fall in ammonia and the accompanying increase in nitrate were also more rapid in the re-steamed soil.

The only effect of the addition of commercial superphosphate after steaming was a slight delay in the fall in ammonia concentration.

The addition of starch immediately after steaming has the effect of reducing the level of ammonia produced and the final nitrate concentration. Within the limits of the experiment these reductions are proportional to the amount of starch added.

Deliberate contamination of the soil after steaming with unsteamed soil has been studied in tomato soil. A difference has been observed in that ammonification in the steamed contaminated soil reached a peak and fell extremely rapidly (11 days) with consequent rapid nitrification, whilst in the steamed soil the ammonia level rose to a higher value and did not fall for some time (28 days) after that in the steamed contaminated soil.

A study of the effect of differing external conditions on the soil after steaming has begun. Samples of three soils commercially steamed in the late autumn were taken immediately before and after steaming. The samples, stored in earthenware pots covered by glass plates to minimise moisture losses, were maintained at approximately 23° C. in an incubator. The contents of the pots were sampled at regular intervals and care taken to minimise possible risk of gross contamination. At the same time samples of soil in the glasshouses were also taken. Here contamination risks were high and the soil subject to temperature and moisture variations and aerial reinfection. Ammonification and nitrification have been followed in the three soils under the above two sets of conditions. Results so far obtained show that the ammonia level has increased and subsequently decreased with an accompanying increase in nitrate in every case in a shorter time in the incubated soils than in those in the glasshouses.

3. THE EFFECTS OF SOME ACUTE MINERAL DEFICIENCIES ON GLASSHOUSE PLANTS

By O. OWEN and J. H. L. MESSING

Most of the work on acute mineral deficiencies has been carried out on chrysanthemums. The following seven varieties were used:—

Sussex Pink
Sussex Bronze
Sussex Red
Rose Harrison
Imperial Pink
Cheshunt White
Market Gold

Cuttings of all the varieties were established in soil in the usual way. Those of Market Gold were transferred to sand on February 23rd and fed with complete nutrient until May 2nd, when the respective deficiency treatments began. The other six varieties were transferred to sand on March 30th and fed with complete nutrient until the various elements were withheld as follows: No manganese and no boron treatments started on June 10th; no magnesium and no calcium on June 30th; and no potash, no phosphate and no nitrogen on July 7th.

The effects of the respective omissions are summarised below.

No nitrogen

The symptoms on the foliage were in general the same as those described last year. These are a loss of colour which develops progressively and eventually to a whole range of yellowish-green to yellow and yellow accompanied by red pigment in the oldest leaves. In Rose Harrison pale-coloured spots appeared on the uppermost leaves. Symptoms appear within two to three weeks in Rose Harrison and Imperial Pink and within five to six weeks in Sussex Pink, Sussex Bronze and Sussex Red after the nitrogen had been withheld from the nutrient solution. The time of reaction of Cheshunt White was intermediate. It is interesting to note that all the varieties show the same general sequences in the development of symptoms irrespective of the time taken for them to appear. A feature is the tendency of young leaves to assume a vertical habit.

One effect of this deficiency on the flowers is that except for Rose Harrison opening is delayed for from three to five weeks. Buds of Rose Harrison opened at about the same time as those of control plants. The diameter of the open blooms is reduced to just over half that of controls, Rose Harrison again being exceptional in that the average diameter was $2\frac{1}{2}$ ins. compared with 6 to 7 ins. in controls. The effect on colour varies. Whereas in Sussex Red and Sussex Bronze the colour was intensified no effect was observed in Imperial Pink and Sussex Pink, while colour in Rose Harrison was somewhat lighter. For all the varieties the petals were firm and the keeping quality was better than in any other treatment, including control.

No phosphorus

Effects on the foliage were apparent more quickly than last year. Stunting was evident but not acute and was followed by characteristic yellowing, browning and dying of old leaves. The order of appearance of symptoms was sensibly the same as in the no nitrogen treatment.

Rose Harrison and Sussex Pink were different from the other varieties in two respects. First, when the older leaves begin to die this proceeds rapidly, whereas in the other varieties this happens comparatively slowly. Secondly, they show no regularity in development of symptoms on individual leaves, while in the other varieties there is a regular sequence in that effects are first seen at the apex of the terminal lobe and then develop along the midrib.

The treatment retards opening of the flower buds, causes some reduction in size of the blooms and is accompanied by a slightly paler colour.

No potassium

The effects were not so pronounced as they were last year, the typical bronzing not being observed until the end of October. This is probably due to the fact that there was more sunshine this year. There was, however, noticeable loss of colour in August and this was least in Cheshunt White and Rose Harrison. These two varieties showed marginal bronzing in contrast to the others, which showed interveinal yellowing.

The size of the blooms was reduced by the treatment, but there were no effects on colour or shape or keeping quality.

Mention must be made of the effects on Market Gold in which treatment started two months earlier than in the other varieties. There was obvious reduction in growth rate within three weeks. Marginal bronzing of the middle leaves was observed in seven weeks and this was followed by distortion of the new growth. Death of leaves commenced in the middle leaves and once it had set in proceeded rapidly both up and down the stems. By mid-September only a few small leaves, still green, remained at the tops of the shoots. By October all the shoots had died together with the very small buds which had been formed.

No calcium

The first effects, namely, loss of colour near the growing points, were manifest in three or four weeks after treatment began. Characteristic dark spots on the foliage of Rose Harrison and Sussex Pink appeared in the fifth and seventh weeks respectively. In the other varieties they did not appear until the tenth and eleventh weeks. About the time of bud formation death of the growing points occurred. Leaves on the flower stems were small and yellow and crowded together owing to the extreme shortness of the internodes. The complete flower stem rarely exceeded 3 ins. in length. Rose Harrison was the first variety to be affected and was closely followed by Sussex Pink and a little later Imperial Pink, Sussex Bronze and Sussex Red reacted together. In Cheshunt White the yellowing is not so intense and veins remain green for a comparatively long time. Bronzing of the edges of the younger leaves, however, does occur.

No magnesium

Here the effects differ widely in the different varieties. Growth generally was but little affected and only slight loss of colour had occurred by the end of August. By November, however, three different types of symptoms had developed.

(1) Interveinal chlorosis was general and this was followed by the development of red tints. The condition is first observed on old leaves which eventually become bronze-coloured and die. Sussex Red and Sussex Bronze conform to this behaviour. In the case of Market Gold the condition was much more pronounced, presumably because the treatment had started earlier, and there was practically no growth of the flower stems and the small buds died without opening.

(2) General loss of colour in the foliage followed by the older leaves turning yellow. Irregular bronze-coloured areas appeared quite quickly and these soon became desiccated. Rose Harrison and Sussex Pink fall into this category and the latter occasionally developed red tints.

(3) A barely perceptible loss of colour took place in the old leaves, some scattered patches of red colouration appearing on only a few of the older ones. Then, in a very short time, the whole of the interveinal areas of both old and middle leaves became covered with small circular brown spots. These spots do not extend but gradually darken in colour and dry out. Imperial Pink behaved in this way.

Cheshunt White does not conform to any of the three categories. In this variety there was a limited loss of colour, some marginal scorch of old leaves and a loss of colour round the edges of the youngest leaves when the flower buds were opening.

In the absence of magnesium, flower buds opened early on all varieties. The size of bloom was reduced, particularly in Cheshunt White, and colour was paler, Rose Harrison being most affected in this respect.

No boron

Here again there are well-defined differences in the effects on the foliage of the different varieties.

(1) Rose Harrison. The effects were shown very early. These consisted of a loss of colour in leaves near the growing points. This condition developed rapidly until the topmost leaves were almost white. Later the older leaves died. No real flower stems were produced and by the end of the season 40 per cent. of the growing tips were dying back. On the remainder, flower buds formed on the tops of tufts of leaves due to the extreme proximity of nodes. Leaves, especially those nearest the buds, acquired a vertical habit.

(2) Imperial Pink, Sussex Pink, Sussex Red, Sussex Bronze and Market Gold. Here yellowing develops at the tops and progresses slowly downward. The internodes are shortened but not so much as in Rose Harrison. As the days shortened flower stems were produced on all these varieties but

they were thinner and shorter than those on control plants. Leaves were small and often malformed and those which were produced late were of a comparatively good green colour, the yellowing being confined to the interveinal areas.

(3) Cheshunt White. Growth rate was reduced quite early, but there was only a slight effect on the colour of the foliage. No flower stems were produced and only one third of the shoots produced flower-buds, the remainder dying back.

The effect of the omission of boron on the blooms is perhaps the most striking outcome of this work. In all varieties some distortion of the ray florets was produced. The first noticeable sign was a lateral rolling of the florets to give almost a tubular effect. This was followed by twisting of the outermost florets. The next stage was curling upwards and inwards longitudinally thus producing, in some cases, incurved blooms on varieties which are normally reflexed. The magnitude of these effects is not the same on all the varieties used. In Sussex Bronze and Market Gold the florets are rolled laterally and the incurving is very pronounced. The size of the blooms is considerably reduced. In Sussex Red there was little rolling, but incurving was marked. This would appear to be similar to that reported by Ferguson and Wright working with the variety Marie Adelaide. In Rose Harrison and Sussex Pink there was definite lateral rolling but only slight incurving. Here the size of the blooms was not so seriously affected. Imperial Pink showed some incurving and rolling. Cheshunt White did not produce a single fully-developed bloom, those which opened being small and malformed. All the coloured varieties showed some loss of colour as the result of the treatment.

Plants in this treatment were the last to produce flowers, the retardation ranging from two to six weeks. Rose Harrison was exceptional in that the blooms were fully open two weeks before those in any other treatment including control.

No manganese

Rose Harrison was the first variety to show any signs of this treatment and they were observed on August 18th as compared with Cheshunt White, which was next, but which showed no symptoms until October 25th.

Rose Harrison and Sussex Pink reacted similarly so far as the actual symptoms are concerned. In both chlorosis of the upper leaves occurred. This was accompanied by light circular spots which eventually turned brown. There was an appreciable amount of interveinal yellowing in the middle leaves. Imperial Pink was rather different in that the whole plant was somewhat lighter in colour and interveinal chlorosis and light brown circular spots appeared on the top leaves at the end of the season. The chlorotic areas later turned brown and dried out. Cheshunt White behaved similarly but the spots were larger and darker in colour and towards the end of the season tended to coalesce into almost black areas. There was no effect on the middle leaves of either of these varieties. Sussex Bronze and Sussex Red showed but slight chlorosis and slight stunting. The early-started Market Gold responded quickly to the treatment and finally was very severely affected. Interveinal yellowing was acute and brown spots appeared on the small leaves near the tops of the shoots.

There was an appreciable delay in flowering and the size of all blooms was reduced. In the case of Rose Harrison buds were formed at the same time as on the control plants, but the appearance of the first ray florets was delayed for five weeks. In other varieties this delay was only a matter of days. The flowers were of indifferent quality, the petals lacking crispness, and they did not keep well.

Grateful acknowledgment is made to Mr. C. J. Horwood, who kindly supplied rooted cuttings of most of the varieties used in these experiments.

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V. GROWERS NOTES

I. TOMATO LEAF-MINER (*Liriomyza solani* Hering)

The Tomato Leaf-miner is a small two-winged fly (*Diptera, fam. Agromyzidæ*) which inserts its eggs into the foliage of the tomato plant in glasshouses. While so doing, the female fly also extracts the sap from large numbers of punctures which it makes upon the leaf surface. The small but conspicuous rounded and discoloured areas thus formed give clear evidence of its presence, but often this is not realised until the extensive and very conspicuous larval mines have made their appearance in the leaves.

Each egg is deposited in a separate pit beneath the leaf epidermis, and the white legless larva, on hatching from it after a period of four to eight days, breaks up the tissues within its reach by means of the dentate mouth-hook. The broken-down plant cells are swallowed and digested, as a result of which a cylindrical mine, partially filled with liquid, is left behind the larva as it progresses always in a forward direction. Except for short periods at the time of the three moults which take place during the larval life of about 10 days, the insect appears to feed ceaselessly day and night. Not before it is ready to pupate does the larva, now about one-eighth in length, break through the epidermis of leaf or stem and make contact with the outer air.

On emergence from the mine, and only rarely does pupation occur within it, the larva may form its hard, yellow to dark brown, barrel-shaped puparium (about one-twelfth inch in length, and one-thirtieth inch in greatest diameter) at the exit hole, or fall onto the upper surface of a leaf beneath, to which the puparium becomes cemented. Much more frequently, however, the larvæ fall purposefully to the ground, and form their puparia shortly after burying themselves beneath the soil surface.

Whatever the conditions of temperature, within ordinary range, prevailing in glasshouses, some flies may emerge from the puparia after a period of a fortnight, but the rate of development within the puparium is so variable, that there exists no possibility of predicting the interval of time which may elapse between the appearance of two successive generations.

While it appears probable that two or even three generations may sometimes be completed annually in glasshouses, it is certain that some flies emerge from puparia, formed at any time of year, only after a period of three months has elapsed, irrespective of conditions of temperature and humidity which may prevail.

There can be no doubt that the sole source of infestation amongst seedlings in propagating houses originates through emergence of flies from the puparia which have overwintered in the soil of glasshouses in which the insect has been present during the previous growing season.

Larval mines have occurred occasionally, and usually sparingly in the cotyledons and lower leaves of cucumber in the early months of the year, and very rarely in the leaves of lettuce during winter, but only in glasshouses in which tomatoes have been infested at the same time or shortly beforehand.

The Tomato Leaf-miner is not recorded in recent lists of British insects: it may have been imported from the Continent and become established upon a small nursery near Edmonton, Middlesex, not long before the year 1935. It now has a wide distribution in glasshouses of the Hertfordshire Lea Valley, but has as yet not been recorded from other districts devoted to the cultivation of tomatoes.

Infestations in tomato houses of the Channel Islands have occurred from time to time, and the insect was described by Hering in 1927 from material obtained from potato and other Solanaceous plants in Germany.

Injury caused by the larvæ

A single female fly, during its life of about a fortnight, may lay as many as one hundred eggs, which it distributes in the leaves of a number of plants over a considerable area. The presence of a few females in a glasshouse will therefore result in the subsequent appearance of larval mines in the foliage of many plants.

In the case of very young seedlings, larvæ which hatch from eggs deposited in the cotyledons may destroy within a period of a few days nearly all the tissues contained in them: in order to complete their development they pass into the main-stem and thence into the rough leaves. Destruction of the cotyledons causes young seedlings to show symptoms of mineral deficiency, and collapse

of the plants may follow as a result of further extensive mining by larvæ in the young rough leaves. Large numbers of seedlings while still in the seed-boxes have sometimes been destroyed in this manner upon commercial nurseries during December and January.

Where older plants, still in the "pot" stage or recently planted out, are implicated, the flies usually select leaflets low down upon the plants in which to deposit their eggs. Although the tissues contained in these leaflets may be destroyed by the larvæ, which later pass within the stem to the upper leaves, it is comparatively seldom that the plant sustains permanent injury. Larval mines occur in the topmost leaves only when the plants are considerably older, and have attained a height of at least 6 ft. In commercial glasshouses, during August and September, cases have been recorded in which almost every leaflet contained a larval mine: in proportion to the whole assimilative surface, however, the comparatively small amount of tissue rendered functionless can hardly be of serious consequence, and even in infestations of such severity, it is doubtful if the loss of crop sustained is appreciable.

Observations on different varieties of tomato and the results obtained from small-scale experiments in glasshouses have indicated that the flies feed upon and lay eggs in the foliage of soft-grown plants, or of varieties which habitually produce a soft type of growth, to a far greater extent than in that of plants with harder and more leathery leaves.

It is possibly more than a matter of coincidence that the insect began to make its appearance upon commercial nurseries in the Lea Valley shortly after the variety *Potentate*, with its luxuriant foliage, was grown practically to the exclusion of all other varieties: shortage of potassic fertilisers during subsequent years may also have contributed to the production of soft foliage favourable to the increase of the leaf-miner.

Preventive measures

The only certain method, as yet known, of preventing infestation of plants in propagating houses is timely resort to steam-sterilisation of the soil, in which overwintering puparia of one or more generations of the insect may have accumulated during the previous growing-season. In several instances, nurseries or portions of them which had previously been infested remained free or almost free from the pest during at least the greater portion of the ensuing growing-season, after steam-sterilisation had been carried out: plants in the glasshouses the soil of which had not been so treated became severely infested.

Though the insect is not of sufficient economic importance to warrant the expense of this practise as a special measure for preventing infestation, the soil of the glasshouse or block of houses which are to be used for the propagation of plants should be steam-sterilised on every nursery upon which infestation might be deemed likely to occur.

Not infrequently, this portion of the nursery has been the only one which has not been steamed. Should feeding-pits made by the female flies be noticed upon the foliage of seedlings, repeated application of powder containing a suitable insecticide throughout the period during which the plants are kept in the propagating house can be expected to minimise the severity of, but not entirely to prevent infestation.

For this purpose, pyrethrum powder applied lightly at four-day intervals would appear to be most suitable, since the application of dusts containing either DDT or BHC has sometimes caused injury to young plants.

A fine deposit upon the leaf-surface of an inert powder such as kaolin, containing no insecticide, prevented the flies from puncturing the leaves with their ovipositors, but when seedlings were dusted with it, a certain, though reduced, number of eggs were inserted into the under-surface to which the powder did not adhere.

The smooth deposit left after application of a spray containing DDT in suspension did not have this effect, and many eggs were inserted into the leaves before the flies had been killed by contact with the insecticide. Puparia have been kept in soil to the surface of which dusts containing either DDT or BHC have been applied: when the flies emerged from them, they were not always killed on coming to the surface and escaping from it. Incidentally, a large proportion of fully-developed larvæ, which were dropped upon soil so treated, buried themselves in it, and formed puparia in which development proceeded normally.

Control measures

In the event of larval mines having made their appearance in the foliage of young plants, still in the pots or in leaflets shortly after planting out in the ground, no good purpose is served by the removal of the leaves or leaflets with a view to destroying the larvæ. Such a practise results in the

destruction of more plant tissue than is devoured by the larvæ during their comparatively short span of life, and in many cases they will either have tunnelled into the stems or leaf stalks, where they will complete their development; or have vacated their mines in order to pupate in the soil.

Young pot-plants in which as many as 20 larvæ have been allowed to complete their mines have, after temporary check, made good recovery.

A large proportion of the larvæ have been destroyed in their mines by a single application of a spray containing about 0.01 per cent. Y-isomer of BHC, i.e. one containing one part commercial BHC in 1,250 parts water. This spray caused no injury to young pot-plants.

Dusts containing BHC, on the other hand, caused very severe injury to plants of this age, though here also the larvæ were killed.

In young plants which had been dipped in a 0.1 per cent. solution of nicotine, only larvæ which had recently hatched from the egg succumbed.

Density of foliage and extent of the area to be covered on commercial nurseries preclude the economic use of insecticidal dusts and sprays upon plants which have attained a height of 5 ft. or more.

No protection from infestation was secured in experiments in which seedlings were raised in soil with which sulphate of potash had been mixed in excess. Owing principally to their unbalanced growth many of the plants collapsed after the larvæ of the miner had hatched from the eggs.

There are indications, however, that sulphate of potash applied as a top-dressing to the soil causes older plants growing in it to produce a type of foliage which is not attractive to the female flies.

Natural control by parasites

The Tomato leaf-miner is subject to parasitism by several different kinds of Hymenopterous and a few Dipterous insects. The most important of these parasites (a Braconid of the genus *Opius*) inserts its eggs into the body of the leaf-miner larva. The larval parasite does not immediately cause the death of its host, which succumbs only after it has formed the puparium either in the ground or more rarely on the surface of a leaf.

Parasites have been observed in propagating houses during the earliest months of the year, and have brought infestations under complete control before the end of June.

The adult parasites are highly susceptible to the effects of insecticides such as DDT and BHC, and it is not improbable that efforts to control the leaf-miner by means at least of DDT, have resulted in an increase rather than a decrease of the pest.

2. NEW INSECTICIDES FOR THE CONTROL OF CARNATION PESTS*

By W. H. READ

Recently introduced insecticides combined with new means of application have profoundly changed many of the older methods of controlling insect pests of carnations grown under glass. New insecticides continue to be discovered and it is possible that some of the substances mentioned in this article will be replaced by more effective insecticides within a comparatively short time. To avoid repetition this article is divided into sections dealing with individual insecticides but before describing these a brief note regarding methods of application may be of interest to those not already acquainted with them.

Aerosols and Smokes

To the scientist an aerosol is a suspension of very minute solid or liquid particles in a gas. In horticulture, however, the term aerosol denotes the mist or fog obtained when a liquid is sprayed into the air of a glasshouse by compressed air or other means which ensures that the particles are so small that they remain suspended in the air for a considerable time. The liquids used for producing insecticidal aerosols are usually solutions of the insecticide in a very volatile solvent.

"Smokes" are the products obtained from the burning of mixtures so compounded that when ignited the greater part of the insecticide is driven off as a cloud of smoke particles.

Considerable research has been undertaken by the manufacturers of smoke generators to devise mixtures which on ignition produce a cloud in which a high proportion of the insecticide remains unchanged and to so regulate the rate of burning that a steady evolution of the insecticide is obtained

without flame. It must not be assumed that the insecticidal action is necessarily due to droplets or particles of the insecticide coming into contact with the insect since in some cases the dispersion of the substance as an "aerosol" or smoke is merely a convenient method for vapourising rapidly sufficient of a substance which is only slightly volatile: the most effective insecticides to use by these methods, therefore, are those which are appreciably volatile.

In this country there are two methods of dispersing insecticides as "aerosols." In one of these, described in the 1946 Report, the solution of the insecticide is sprayed into the air of the glasshouse with a compressed air operated paint-spray-gun. The other method is by using a proprietary apparatus in which the solution of the insecticide is expelled as a mist by the pressure derived from a small cartridge of compressed carbon dioxide. The liquified gas aerosol generators employed in the United States of America consist essentially of a solution of the insecticide in a liquified gas held under pressure in a metal cylinder. When released by a trigger valve the pressure forces the solution through a nozzle and the expansion of the gas breaks up the solution into minute droplets.

In this country generators for the production of insecticidal smokes usually consist of small metal containers filled with a mixture of the insecticide and other materials which, when ignited, burns steadily at a low temperature without flame and emits the insecticide as a mixture of smoke and vapour. Other types are available: some consist of blocks of compressed pyrotechnic mixtures whilst simpler forms resembling the well-established nicotine smoking preparations are also employed for some insecticides.

The great advantage of "smokes" compared with "aerosols" is that the operator need not be exposed to the insecticide since by the use of slow-burning fuses it is possible to be well away from the generator before the insecticide is evolved. The very dangerous nature of some insecticides renders them unsuitable for "aerosol" application under commercial conditions.

Azobenzene

This insecticide may be employed in "aerosols" and "smokes" for the control of the glasshouse red spider. Carnations are very tolerant of azobenzene and normally there is ample space above the plants to enable aerosols to be used without fear of hitting them with droplets of liquid, and for smokes to disperse so that excessive local concentrations are avoided. It is therefore possible to use dosages of 8 grams of azobenzene to each 1,000 c. ft. of glasshouse capacity and obtain satisfactory control provided a second application is given eight to ten days after the first to destroy eggs laid by adult females which may survive for several days after treatment with azobenzene. The only disadvantage attached to the use of azobenzene is the necessity of keeping the house shut for at least four hours, preferably six hours, at a temperature of 70° F. In hot weather it is best to carry out the treatment in the late afternoon or evening and leave the houses closed overnight.

HETP and TEPP

These are the initials given to the two products hexaethyltetraphosphate and tetraethyl-pyrophosphate. Recent investigations have shown that the insecticidal properties of HETP are almost entirely due to its tetraethyl-pyrophosphate content which is about 20 per cent. in most commercial samples. The tetraethyl-pyrophosphate content of commercial TEPP is about 40 per cent., consequently TEPP is approximately twice as powerful as HETP.

These insecticides are unsatisfactory for aerosol, smoke, or dust distribution and consequently they must be applied as sprays. Whilst extremely poisonous when taken orally or absorbed through the skin they are readily destroyed by the action of moisture and become virtually non-poisonous after 48 hours. Foliage sprayed with them at the concentration required to control the pests against which they are effective may be handled after 24 hours.

Obviously spraying dilutions must be used without delay and the concentrate kept so that no moisture can gain access.

Sprays containing one part of pure tetraethyl-pyrophosphate in 10,000, equivalent to one part in 2,000 of commercial HETP or one in 4,000 of commercial TEPP are of value for the control of the green peach aphid (*Myzus persicae*) when used with an efficient wetting agent. The eggs of the red spider are not affected, however, and since these sprays have no residual action two or three sprayings at intervals of 10 to 14 days are necessary.

The experience of commercial carnation growers has confirmed that spraying with these insecticides, gives better control of aphids than is obtained with one in 1200 nicotine and provided a satisfactory wetting agent is employed the penetration by the fluid of the folded leaves of the growing tip yields more satisfactory results than are given by BHC smokes.

Thrips are destroyed by HETP and TEPP when exposed to the spray, but DDT and BHC are superior for controlling this insect on account of their persistence.

DDT

Thrips tabaci and the caterpillars of the carnation tortrix (*Cacæcia pronubana*) are very readily controlled by DDT as are some pests of lesser importance such as earwigs and caterpillars of the Tomato moth (*Diataraxia oleracea*).

For this purpose DDT dusts, sprays, smokes or aerosols may be used. Dusts are the cheapest form in which to apply DDT to carnations and provided an efficient dusting appliance is available give very effective control. The quantity of dust required is small and barely visible on the foliage if properly applied by blowing it into the air above the plants and allowing it to settle. Fifteen pounds of a 5 per cent. dust suffices for one application to an acre of carnations if carefully distributed. DDT is without appreciable effect on aphids and red spider mites are unaffected.

Benzene hexachloride—B.H.C.

The active principle in benzene hexachloride, the gamma isomer, has been named Lindane, but many will be more familiar with the proprietary name "Gammexane."

Benzene hexachloride is somewhat similar in its insecticidal properties to DDT and may also be obtained in the form of spraying preparations, dusts and smoke generators. It is less effective than DDT against caterpillars but will control aphids. Dusts are unsatisfactory for this purpose, however, since they do not reach aphids within the leaves of the growing tips and smokes often give only partial control on this account. Good control of thrips is obtainable with dust, however. Where carnation beds are made up with turf in which there may be wireworms, treatment of the soil before planting by raking a BHC dust into the surface soil will prevent loss of plants. A dust containing 2 per cent. commercial BHC should be used at the rate of $\frac{3}{4}$ oz. per sq. yd.

Parathion

This is the name given to *para*-nitrophenyl diethyl thiophosphate, which was referred to as E 605 in earlier Reports. Parathion is very effective against a wide range of insects and particularly against aphids and red spider. It is more volatile than TEPP and is effective when dispersed as a smoke or aerosol, but owing to its extremely poisonous nature it cannot safely be commercially employed in the latter process. Although more volatile than TEPP it is nevertheless more persistent on treated foliage on account of its stability in the presence of moisture. Whilst the immediate danger when applying parathion can be avoided by using smokes and employing one person to each house in a block when lighting the fuses of the smoke generators, the danger of deposits on the foliage cannot yet be assessed since no information is available on what constitutes an injurious deposit having regard to the nature of the foliage and extent to which it is handled. Parathion is readily absorbed through the skin and many workers elsewhere have been injured by contact with foliage sprayed with parathion more than 10 days previously. Furthermore, there is a possibility that some of the parathion may be changed to even more dangerous substances by the heat within the generator.

The active stages of red spider which are tolerant to azobenzene are killed under commercial conditions by dispersing half a gram of parathion as a "smoke" to each 1,000 cu. ft. of glasshouse capacity. The use of combined azobenzene-parathion smokes in which the azobenzene destroys the eggs and parathion the active stages gives an excellent control of this pest. Most of the resting stages are destroyed by this combination but as half a gram of parathion per 1,000 cu. ft. does not yield sufficient residue on the plants to destroy the active mites which emerge from unkilld resting stages it is necessary to repeat the treatment in two days' time in hot weather or three days' time in cooler weather, in order to obtain a complete control.

Although this quantity of parathion will produce a smaller deposit on the foliage than that resulting from spraying with 0.01 per cent. parathion, no information is yet available to say whether the amount of deposit is so low that it is quite safe for workers to handle plants either immediately after treatment or within any stated period. In view of the dangerous nature of parathion it is advised that other insecticides be used until parathion has been proved safe. If parathion products are used every precaution advised by the manufacturers must be strictly observed, including the provision of adequate ventilation of the houses and avoidance of handling plants for a considerable period after treatment.

Systemic insecticides

A systemic insecticide is one which can be absorbed through the roots or foliage and conveyed in the sap throughout the whole, or greater part, of the plant. The dangers of sodium selenate due to its persistence in the soil are such that its use is most inadvisable. Experiments made with more recently discovered systemic insecticides indicate that one of these bis-(bis-dimethylamino) phosphonous anhydride will control red spider and the green peach aphid when sprayed on the foliage or watered on the soil. More work is necessary to determine the best conditions under which to use it and the effect of repeated applications on the vigour and flower production of plants.

Summary

This paper gives a popular review of the uses of some of the newer insecticides for the control of insect pests of carnations under glass.

* This is a modified version of an article appearing in the "Carnation Year Book 1949," with a few additions.



APPENDIX "A"

PUBLICATIONS

1919

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APPENDIX "B"

MANURIAL TREATMENT IN TOMATO EXPERIMENTS

(Except plots in Appendix C)

House	Plot	BASE TREATMENT						TOP DRESSING								
		Sterilisation	Lime	Stable Manure	Hoof and Horn	Bone Meal	Sulphate of Potash	Sulphate of Ammonia	Sulphate of Potash with first Watering	Balanced Mixture	Dried Blood Mixture	Sulphate of Ammonia	Superphosphate	Sulphate of Potash	Dried Blood	Nutrient Feeding by Solu-feeder
1	1	Steam	1 ton per acre		10 cwt. per acre	10 cwt. per acre	10 cwt. per acre		5 cwt. per acre	6 applications	None					
		Steam	1 ton per acre		10 cwt. per acre	10 cwt. per acre	10 cwt. per acre		5 cwt. per acre	6 applications						
3	1	"	"		"	"	"		"	"						
	2	"	"		"	"	"		"	"						
4	3	"	"		"	"	"		"	"						
	4	"	"		"	"	"		"	"						
5	5	"	"		"	"	"		"	"						
	6	"	"		"	"	"		"	"						
7	7	"	"		"	"	"		"	"						
	8	"	"		"	"	"		"	"						
8	1	Formaldehyde	1 ton per acre	None	10 cwt. per acre	10 cwt. per acre	10 cwt. per acre		5 cwt. per acre	7 applications						
	2	Formaldehyde	1 ton per acre	None	10 cwt. per acre	10 cwt. per acre	10 cwt. per acre		5 cwt. per acre	None						
9	3	"	"		"	"	"		"	"						
	4	"	"		"	"	"		"	"						
10	5	"	"		"	"	"		"	"						
	6	"	"		"	"	"		"	"						
10	7	Formaldehyde	1 ton per acre	None	10 cwt. per acre	10 cwt. per acre	10 cwt. per acre		5 cwt. per acre	None						
	8	Formaldehyde	1 ton per acre	None	10 cwt. per acre	10 cwt. per acre	10 cwt. per acre		5 cwt. per acre	6 applications						
10	9	"	"		"	"	"		"	"						
	10	"	"		"	"	"		"	"						

APPENDIX "C"

MANURES APPLIED IN PLOTS 1-3 AND 8-10 IN TOMATO HOUSE 4

Plot	Title	Base Manure in Lbs. per sq. yds.				*Top Dressing in Lbs. per sq. yds.		
		Lime	Stable Manure	Super- phos- phate	Sulphate of Potash	Super- phos- phate	Sulphate of Ammonia	Sulphate of Potash
1	Complete Artificials (I) less Nitrogen	1.0	—	0.4	0.1	0.5	—	0.2
2	Unmanured	—	—	—	—	—	—	—
3	Complete Artificials	1.0	—	0.4	0.1	0.5	0.2	0.2
8	Complete Artificials with Dung	1.0	7	0.4	0.1	0.5	0.2	0.2
9	Complete Artificials less Phosphate	1.0	—	—	0.1	—	0.2	0.2
10	Complete Artificials less Potash	1.0	—	0.4	—	0.5	0.2	—

* The Top-dressing was applied in eight equal instalments.

APPENDIX "D"

DATES OF CULTURAL OPERATIONS, 1949

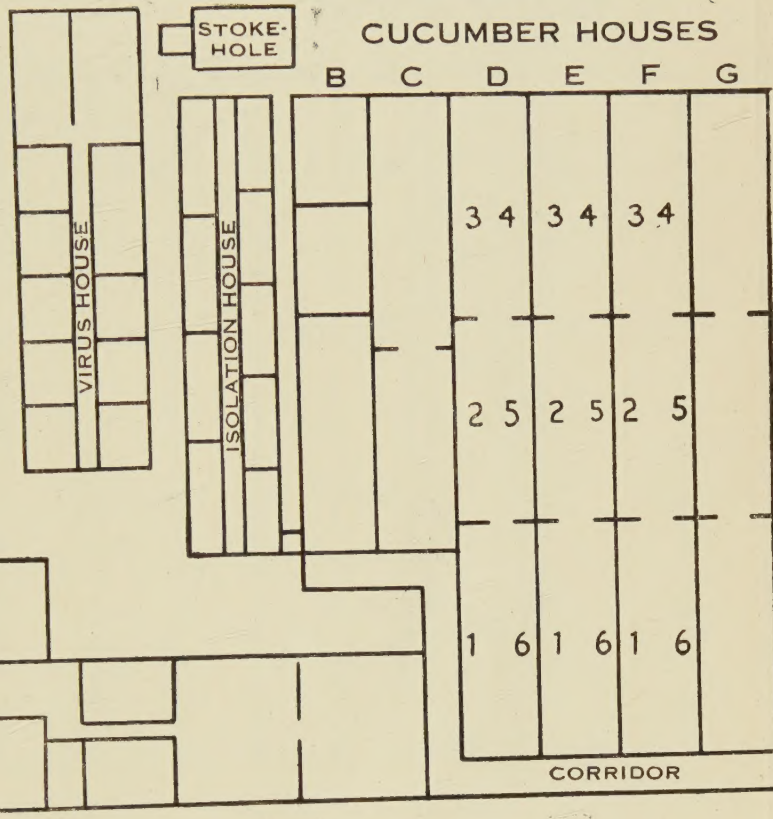
Crop	* Sown	Potted into 60's	Potted into 32's	Planted out in Borders	First top Dressing	First Fruit Picked	Last Feed Applied	Last Fruit Picked
Tomatoes	Jan. 1	Jan. 24	—	Mar. 8	May 11	May 24	Aug. 23	Oct. 16
Cucumbers	Jan. 21	—	Feb. 9	Feb. 25	Apr. 2	Mar. 28	Aug. 13	Sept. 6

APPENDIX "E" METEOROLOGICAL RECORDS, 1949

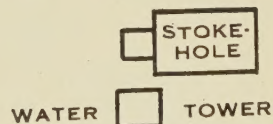
Month	Air Temperatures						Grass Temperatures				Rainfall				Mean Soil Temperature		Hygrometer				Bright Sunshine		Barometer
	Mean			Absolute Maximum			Absolute Minimum			Av. Min.	Total			No. of Days	Greatest Fall		Dry Bulb	Depress. of Wet Bulb	Relative Humidity	Dew Point	Total No. of Hours	Daily Average	
	Temp.		Date	Temp.		Date	Temp.		Date		Precipitation	Amt.	Date		° F.	° F.							
	Max.	Min.		° F.	° F.		° F.	° F.															
JANUARY	46.3	35.0	53.0	14th	25.0	29th	32.1	21.0	29th	1.00	9	0.36	3rd	41.1	41.4	1.2	90.3	38.7	38.0	1.2	—		
FEBRUARY	51.0	33.9	57.0	16th 18th 20th 21st 23rd	18.0	3rd	30.1	15.0	3rd	1.16	5	0.57	8th	39.1	41.7	1.4	86.9	38.0	96.5	3.4	—		
MARCH	49.6	34.4	67.0	26th 27th	26.0	11th 19th	32.0	22.0	19th	0.80	5	0.30	4th	40.9	43.1	2.1	84.8	38.6	94.4	3.0	29.81		
APRIL	63.5	41.7	84.0	16th	30.0	8th	37.5	25.0	8th	1.31	9	0.35	28th	48.4	52.8	3.5	78.3	45.9	190.5	6.3	29.66		
MAY	63.3	43.7	72.0	22nd	31.0	9th	41.0	26.0	9th	1.98	12	0.79	23rd	51.2	53.4	2.8	82.5	48.0	202.3	6.5	29.62		
JUNE	72.5	50.2	89.0	27th	38.0	1st	46.4	36.0	1st	0.43	5	0.25	1st	58.4	62.2	3.5	80.4	55.7	221.9	7.4	29.79		
JULY	77.8	54.1	89.0	12th	45.0	7th 19th	47.1	38.0	7th	1.49	7	0.90	17th	61.8	67.3	1.6	90.5	64.3	218.4	7.0	29.78		
AUGUST	76.7	53.0	88.0	15th	43.0	11th	45.1	34.0	11th	1.50	6	0.86	1st	59.9	65.0	2.3	87.1	60.8	229.4	7.4	29.72		
SEPTEMBER	72.7	54.3	87.0	5th	43.0	17th	46.3	36.0	17th 18th	0.17	4	0.08	21st	60.0	63.2	2.1	87.9	59.4	143.3	4.8	29.68		
OCTOBER	63.7	44.2	80.0	3rd	27.0	27th	38.8	25.0	27th 30th	5.14	13	0.94	23rd	53.9	53.5	1.9	87.9	49.7	118.0	3.8	29.66		
NOVEMBER	50.0	37.2	59.0	4th	26.0	1st	34.6	23.0	1st	2.49	15	0.54	5th	44.3	42.9	1.3	89.7	40.0	58.9	2.0	29.44		
DECEMBER	48.5	36.1	56.0	3rd 4th	26.0	11th	29.7	22.0	11th	1.37	14	0.37	18th	42.6	42.3	1.5	87.5	38.8	43.7	1.4	29.50		

Total Rain for Year=18.84 inches. Number of Days Precipitation=104. Total Bright Sunshine for Year=1655.3 hours.

DIAGRAM SHEWING ARRANGEMENT



No. 1	
8	9
7	10
6	11
5	12
4	13
3	14
2	15
1	16



ENGINE HOUSE

OF EXPERIMENTAL PLOTS, 1949

TOMATO HOUSES

